QH
.R338
vol. 65
no. 1-2

Transactions

of the

Illinois State Academy of Science

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NEW YORK
BOTANICAL GARDEN

Volume 65
Nos. 1 and 2
1972

Springfield, Illinois

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Mailing date April, 1972

38364—1650—2-72

TRANSACTIONS

OF THE

ILLINOIS STATE ACADEMY OF SCIENCE

VOLUME 65 - 1972 Nos. 1 and 2

ILLINOIS STATE ACADEMY OF SCIENCE

AFFILIATED WITH THE

ILLINOIS STATE MUSEUM DIVISION

SPRINGFIELD, ILLINOIS

(PRINTED BY AUTHORITY OF THE STATE OF ILLINOIS)

RICHARD B. OGILVIE, *Governor*

March 1, 1972

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INDUCTION OF GROSS DELETIONS IN RING AND ROD X CHROMOSOMES OF DROSOPHILA

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ABSTRACT.—A comparison was made of induction by X-rays of gross deletions of ring and rod X chromosomes in the mature spermatozoa of *Drosophila melanogaster*. The deletions were detected by the attached X method. There were no differences between the chromosomes in 0-2 and 2-4 day broods; however, in 4-6 day broods which represented spermatids and spermatocytes at the time of irradiation there was a significant increase in deletions in the ring chromosome compared with the rod chromosome. The extra amount of heterochromatin or the ring nature of the chromosome may be the responsible factors in the increase in gross deletions.

For many years the induction of sex-linked recessive lethals in the ring chromosomes of *Drosophila melanogaster* were thought to be "point mutations" rather than deletions or minute rearrangements (Muller, 1940; Sobels, 1964). Recently Falk (1970) reported that most of the lethals induced by radiation in the X chromosome were deletions and that fractional recessive lethals were induced twice as often in a ring X chromosome as compared to a rod X chromosome. If chromosome aberrations are indeed induced more frequently in the ring chromosome, then gross deletions of the X chromosome induced should be higher in the ring chromosome.

To test this hypothesis, male Drosophila containing the ring X^{c2} chromosome with the mutant genes *ec* (echinus) and *f* (forked) were irradiated with 2000R of X-rays produced by G. E. Maximar III-250 unit at 150Kv, 15 ma, at 35 cm. Oregon-R males were used to obtain information on the rod X chromosome. The three day old males were then mated to females with attached X chromosomes, containing the following mutants *y* (yellow) *v* (vermillion) *f* (forked) *car* (carnation) at a ratio of one male to two females. The viable offspring are males which received the X chromosome from their father and the Y from the mother, and the females were normally produced by an egg with the attached X fertilized by the Y containing sperm from the father. The triplo X and the YY offspring die, however if the X chromosome from the irradiated male is small due to a gross deletion, the zygote will survive with the attached X chromosomes. The deleted X with the wild type alleles will mask the recessive mutant genes on the X chromosome and these exceptional females can readily be detected.

The irradiated males were transferred every two days to a new group of females until day six after X-ray exposure. By this brooding technique information was obtained on the damage to various post meiotic stages of spermatogenesis. The offspring of 0-2 day mating resulted from mature or almost mature spermatozoa at the time of irradiation. The 2-4 day brood represented immature spermatozoa and late spermatids while 4-6 day broods yielded information on early spermatids and spermatocytes.

There was no significant differences between the percentage of induced deletions in ring and rod X chromosomes in broods 0-2 and 2-4 days (Table I). However, in 4-6

TABLE 1.—Deleted X induced in rod and ring X chromosomes.

Brood Period	Rod deletions	Total	Percentage	Ring deletions	Total	Percentage
0-2 days	2	1212	.165	3	1162	.258
2-4 days	3	1280	.234	5	1261	.397
4-6 days	1	654	.155*	3	395	.759*

*$\chi^2 = 10.3$, 2x2 contingency table with Yates' correction.

day broods (Table I) there was a significant increase of deletions in the ring chromosome. It may be the larger proportion of heterochromatin in the ring chromosome or the shape of this chromosome that yields to more breakage by radiation. Catcheside and Lea (1945) reported more inversions, deficiencies and intercalations in ring than rod chromosomes although the loss of many of the aberrations in the ring chromosome occured by torsional rejoining. Although the ring X chromosome is more sensitive to loss due to X-ray treatment, the increase of induced deletions in the spermatids with the ring chromosome represented a much larger percentage of breakage and rejoining than was evident. There is indeed quite a difference between recessive sex-linked recessive lethals and gross deletions and it is not proper to compare them too closely; although both aberrations represent a breakage, and loss of part of the chromosome, there is a difference in the size of the deletion. The lethals are usually minute deletions and are produced and recovered five times as frequently as gross deletions by the attached X method. The data presented here indicated that more deletions were produced in the spermatids of the ring X chromosomes as compared to rod and that there was no difference with respect to gross deletions in later stages of spermatogenesis. The extra amount of heterochromatin or the position of the ring chromosome may be the responsible factors for the increase in gross deletions.

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Manuscript received August 5, 1971

PITH CONDITION RATING SYSTEM FOR SUDAN GRASS STEM TISSUE

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ABSTRACT.—Yields and pith condition ratings based on areas of white, spongy tissue in the stem were compared in wind-lodged sudan grass plants grown in 18 variations of nitrogen and phosphorus fertilization. In general, as nitrogen and phosphorus fertility was increased, yield and pith condition ratings increased.

Sudan grass is used for pasture, hay and silage in the corn belt. It is an annual grass sorghum with slender stems. In a recent trial to study the effect of fertility on growth and yield, severe lodging occured following a rain and wind storm. Although the plants showed no symptoms of crown or root rot, some differences in stem pithiness were noted. We decided to use the pith condition rating system (based on the percent dead, white parenchyma in the internode) devised for studies of parenchyma cell death in sorghum (Katsanos and Pappelis, 1969), corn (Pappelis, 1970) and sugar cane (Pappelis and Katsanos, 1965) to determine whether any differences in pithiness could be detected and attributed to fertilizer treatments.

The experiment was conducted on the Southern Illinois University Belleville Research Center, Belleville, on Ebbert silt loam soil (O.M. -2%). Before planting the sudan grass (*Sorghum vulgare* Pers. var. Piper), 200 Kg/ha K_2O was broadcast and disked into the experimental area. The four replicates were planted June 8, 1967, at the rate of about 22 Kg/ha in 2.4 X 9 m plots, drilled in 22.5 cm rows. Plants emerged June 13.

The rating system for pith tissue was: 0.0, no white tissue in the internode longisection (cut through the central axis); 0.1, 1% of the internode white; 0.5, 2 to 12% white; 1, 13 to 25% white; 2, 26 to 50% white; 3, 51 to 75% white; and 4, 76 to 100% white. The elongated internodes of ten plants per replicate were rated in each treatment plot by cutting the plants through the roots to remove them from the plot and cutting the stem longitudinally through the central axis to expose the internode tissue for pith condition rating.

About 95 to 100% of the plants in all treatments except those in control plots (only fertilized with K_2O) lodged on the evening of July 9 following a relatively intense but brief windstorm. The plots had received about 1.2 cm of rain between 6 and 10 AM that morning. All lodged plants were fully erect by July 12. The heights of the plants at the time of lodging were about 90 cm. The maximum temperatures for July 9 through 12 were 28, 33, 33, 33, and 30°C, respectively, and the minimums were 20, 14, 20, and 20 respectively. The pith condition ratings were performed on July 14.

The harvesting data (average of four replicates) in metric tons per hectare, adjusted to 70% moisture, for the first cutting made July 25, are given in Table 1. The least yield was measured in the control plots (0-0-200 Kg/ha N P K, respectively), 15.1 mt/ha. We considered the six treatments yielding 19.4 mt/ha or above as being signi-

TABLE 1.—Pith condition ratings of four elongated internodes of the lower stem (average of 10 plants per plot per replicate) and first cutting yields as metric tons per hectare (mT/ha) of sudan grass var. Piper (average of four replicates) grown in Ebbert silt loam with fertility treatments which include 180 lb/A muriate of potash (K_2O) and various amounts of urea or ammonium nitrate (AmN) with or without ammonium phosphate (AmP), diammonium phosphate (DiAmP), ammonium polyphosphate (AmPP), or triple super phosphate (TSP).

Nutrients Kg/ha	Internodal Pith Condition Ratings				Yield mT/ha
	1	2	3	4	
200 Kg/ha K_2O					
Plus no other	2.4	2.7	2.2	0.2	15.1
Plus 67 Kg/ha TSP	2.5	2.6	2.4	0.3	17.2
Plus 67 Kg/ha AmN	2.4	2.7	2.0	0.4	16.3
Plus 67 Kg/ha Urea	2.5	2.4	1.3	0.2	14.3
200 Kg/ha K_2O and 67 Kg/ha AmN					
Plus 67 Kg/ha DiAmP	2.3	2.7	2.1	0.5	18.1
Plus 134 Kg/ha DiAmP	2.7	2.7	2.6	0.7	19.6
Plus 134 Kg/ha AmPP	2.3	2.8	2.2	0.5	17.2
200 Kg/ha K_2O and 67 Kg/ha Urea					
Plus 67 Kg/ha AmP	2.6	2.8	2.2	0.3	18.5
Plus 67 Kg/ha DiAmP	2.8	2.9	2.5	0.6	18.1
Plus 67 Kg/ha AmPP	2.7	2.9	2.5	0.8	18.7
200 Kg/ha K_2O and 134 Kg/ha Urea					
Plus 67 Kg/ha AmP	2.7	2.8	2.3	0.6	20.2
Plus 67 Kg/ha DiAmP	2.4	2.6	2.3	0.5	19.4
Plus 134 Kg/ha AmP	2.7	2.8	2.4	0.7	18.1
Plus 134 Kg/ha DiAmP	2.6	2.7	2.2	0.7	20.2
200 Kg/ha K_2O and 134 Kg/ha AmN					
Plus 67 Kg/ha DiAmP	2.7	2.8	2.5	0.8	16.9
Plus 67 Kg/ha AmPP	2.6	2.9	2.4	0.7	18.3
Plus 134 Kg/ha DiAmP	2.7	2.9	2.4	0.7	19.8
Plus 134 Kg/ha AmPP	2.0	2.8	2.9	2.5	23.8

ficantly greater than the others. Of these six, the greatest yield was with the highest amount of ammonium polyphosphate (134-134-200), 23.8 mt/ha.

The pith condition ratings (average for four replicates) are given in Table 1. Generally, three to four internodes had elongated. The most advanced growth was observed in plants from the ammonium nitrate plus ammonium polyphosphate treatment plots (134-134-200 Kg/ha) which also produced the highest yield. If diseased crown and stem tissue had been present, the stalk rot rating system used for corn (Pappelis, 1970) would have been appropriate.

In general, as N and P were increased, yield and cell death in stem tissue (pith condition ratings) increased. This is in agreement with the effects of these elements on parenchyma cell death in corn stalk tissue (Pappelis and Boone, 1966). Whether similar trends would be found with less fertile soil and a wider range of fertility remains to be determined.

ACKNOWLEDGMENTS

This research was supported in part by National Science Foundation Grant GB5559, the Tennessee Valley Authority Grant to Southern Illinois University Belleville Research Center, and Interdisciplinary Research in Senescence, a cooperative Research Project of Southern Illinois University.

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Manuscript received March 1, 1971.

NUCLEAR DRY MASS AND NUCLEAR AREA OF HUMAN BUCCAL MUCOSA CELLS

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ABSTRACT.—The averages for nuclear dry mass (NDM) and area (NA) for 80 nuclei of human buccal mucosa cells (doner: G.A.P., age 13, male) were 73.1 x 10^{-12}g and 83.1 x 10^{-8}cm^2, respectively. The NDM and NA means for 10 replicates were not significantly different at the 5% level, suggesting that smaller sample sizes are adequate to estimate the population NDM and NA means.

After learning to operate the Leitz Transmitted Light Interference Microscope, I did a study of the nuclei of my buccal mucosa cells, studying 8 nuclei from each of ten smears. I calculated nuclear area (NA) by photographing the nuclei with a Leitz Orthomat camera, developing the film in Diafine, tracing the projected images, and determining the area of the tracing using a planimeter. The magnification constant was determined using the projected image of a stage micrometer. The nuclear dry mass (NDM) for each cell studied was calculated using the optical path difference (OPD) for nuclei with with monochromatic light (546 nm), the specific refractive index for protein (=0.18), and the NA in a formula described by G. Beneke (1966):

$$\text{NDM (in grams)} = \frac{\text{OPD} \times \lambda \times \text{NA.}}{\chi}$$

The NDM average for 80 cells was 73.1 x 10^{-12}g and for NA, 83.1 x 10^{-8}cm^2, with the individual replicate averages ranging from 51.5 to 91.6 x 10^{-12}g for NDM from 72.3 to 100.2 x 10^{-8}cm^2 for NA. The ranges for individual NDM was from 14.7 to 120.9 x 10^{-12}g and for individual NA, 36.1 to 140.5 x 10^{-8}cm^2. I then compared the ten average NDM and NA scores (Table 1) against each other, using the analysis of variance test, randomly selecting 5 scores as the "treatment" scores and the remaining 5 as the "control" scores. In two such tests, I found no significant difference between the treatment and control groups at the 5% level, indicating little change between samples taken on different days. This meant that a smaller number of replications could be adequate to obtain a good estimate of the population mean.

TABLE. 1.—Nuclear dry mass (NDM) and nuclear area (NA) for G.A.P. buccal mucosa smears. Each replicate is the mean of 8 cells per cell.

Smear	NA[1]	NDM[2]
1	87.8	67.8
2	84.7	84.3
3	72.3	51.5
4	79.6	73.0
5	72.4	74.4
6	86.8	91.0
7	85.8	69.2
8	100.2	82.0
9	79.1	61.2
10	81.7	75.6
Average	83.1	73.1

1. NA is x 10^{-8}cm^2
2. NDM is x 10^{-12}g

My results for NDM and NA compared favorably with other studies of these nuclear characteristics in buccal mucosa cells; however, variations did exist. Sandritter *et al.* (1958) reported the average NDM for 38 cells to be 55.5 x 10^{-12}g and NA to be 87.0 x 10^{-8}cm^2, while more recently, Schiemer and Lanschke (1970) reported the average NDM of 800 cells to range from 39.9 to 53.3 x 10^{-12}g. In their paper, they also cited other work in which the average of 5000 cells was about 39 to 43 x 10^{-12}g. The

sex and age of the donor(s) were not given in any of these studies. Of the 10 replicates for NDM in my study, only one is in the range cited by Schiemer and Lauschke (1970).

Since I found the 10 replicate means for NDM and NA to be quite different, although not statistically different, I thought that the results could be interpreted as an age related difference, since the average NA in my study is almost identical to that reported by Sandritter *et al.* (1958) and Niebergs (1967) while the NDM mean for my cells is greater than that reported by the other published works cited above. I assumed that since no ages were given, their donor(s) were possibly older than I, both physiologically and chronologically.

When the variation due to sampling was considered, possibly due to physiological status on the various sample days, the mean for the 4 lowest replicates for NDM in my study (36 cell total) was more like that reported by Sandritter *et al.* (62.8×10^{-12}g) and the NA remained in good agreement (81.4×10^{-8}cm^2). Although Koss (1968) and Niebergs (1967) report that cell size and nuclear size can increase due to various health related factors ranging from vitamin deficiency to cancer, it may be that these variables could be extended to age associated differences. Thus, it may be significant to report sex, age, and general health status of each donor in future NDM and NA studies.

Acknowledgments

I wish to thank the following: Mrs. Zoe Lightfoot, Science teacher, Carbondale Community High School, for permitting me to do work outside my ninth grade class, for which this report was prepared; Mr. Joseph Karmos, Mathematics instructor, Comprehensive School Mathematics Program—Central Midwestern Regional Educational Laboratories, Inc., who guided my studies in statistics and computer training during that same period; A. J. Pappelis and W. S. Courtis for my training on the Leitz Interference Microscope and related equipment; and C. C. Lindegren for the Leitz Interference Microscope (NIH Training Grant number 5 TO1 GM 00593-08 from the National Institute of General Medical Science).

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Manuscript received July 12, 1971.

THE EFFECT OF LIGHT QUALITY UPON PHENYLALANINE AMMONIA-LYASE (PAL) ACTIVITY IN POTATO TUBER TISSUE SLICES

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ABSTRACT.—Phenylalanine ammonia-lyase (PAL) was extracted from both fresh and aged slices of potato tuber tissue. Using modified purification procedures, the PAL removed from potato exhibits an absolute requirement for Mg^{++} in order to obtain activity. The induction of increased levels of PAL is partially light dependent. No action spectrum was found.

Phenylalanine ammonia-lyase (PAL) catalyses the conversion of L- phenylalanine to trans-cinnamic acid. This product may then be converted by various metabolic steps into coumarin, caffeic acid, chlorogenic acid, or lignin. Since the original observation by Nutile (1945) that light could reverse the inhibition of seed germination by coumarin, numerous investigations have been concerned with the effect of light upon the activity of this enzyme. Particular attention has been focused on the involvement of phytochrome in the control and distribution of PAL activity (Attridge and Smith, 1967; Mohr et al., 1968; Bellini and Van Poucke, 1970).

Few investigations have been concerned with the level of PAL activity in storage slices. Zucker (1965) has shown that the production of chlorogenic acid is directly related to the production of the enzyme in potato and that the level of enzyme activity is light dependent. Studies using cycloheximide indicate that a *de novo* synthesis of the enzyme is involved (Zucker, 1968).

This paper shows that PAL isolated from potato tubers requires Mg^{++} for activation. Data are presented which show that the activity of the enzyme increases in physiologically aged tissue slices and that this level is enhanced by red, far-red, blue, or white light.

METHODS AND MATERIALS

Slices 8.0 x 1.0 mm were prepared from Russet white potatoes using a hand microtome. They were immediately placed in cold distilled water and were kept chilled until the initiation of the aging process. Aging was carried out at room temperature in aeration chambers fitted with Cinemoid light filters (Century Lighting, New York). The aging solution consisted of 60-0 ml of 10^{-4} M $CaSO_4$ which was renewed twice during the 24 hr aging process. Each chamber contained 120 slices, approximately 7.5 g.

TABLE 1.—*The Spectral Ranges, Light Intensities, and Length of Exposure Used to Promote PAL Synthesis.*
Except for the exposure to white light, tissue irradiation was applied at 1 hr of aging.

Light Regime	Wavelength Range (nm)	Intensity (ergs cm^{-2} sec^{-1})	Dose (ergs cm^{-2})
Red	590-700	$4.0.10^2$	(2-1/2 min) $6.0x10^5$
Far-Red	730-800	$5.4.10^4$	(1 min) $3.2x10^6$
Blue	380-550	$2.5.10^2$	(2-1/2 min) $3.8x10^5$
White (Fluorescent)	370-750	$4.4.10^3$	24 hr

The experimental tissue was allowed to age 1 hr before being illuminated. Table 1 indicates the wavelengths, intensities, and doses of irradiation employed. Two controls, one in complete darkness, and the other in continuous fluorescent white light (100 ft-c), were also used.

Phenylalanine ammonia-lyase was isolated using a modification of Rissland and Mohr (1967). The 120 slices were homogenized in the cold and dim light for 5 min in the presence of 20.0 ml of 0.1 M borate buffer, pH 8.8, 0.4 ml sulphite mixture (Verleur and van der Weyden, 1969), and $2x10^{-3}$ M $MgCl_2$. The homogenate was filtered through cheesecloth and centrifuged at 34,000 x G and -1 C for 25 min. The supernatant was then mixed for 10 min with 1.0 g of activated charcoal for every 5.0 ml of solution and recentrifuged as above. The resulting supernatant was then passed through #41 filter paper, the filtrate containing the enzyme. This enzyme solution was stable, losing only 9% of its activity after 1 day at 0 C.

The assay mixture consisted of 200 umoles of borate buffer, pH 8.8, 60 umoles of L-phenylalanine, $2x10^{-3}$ M $MgCl_2$, and 1.0 ml of enzyme extract (400-600 ug protein). The final volume was adjusted to 6.0 ml. One unit of enzyme activity was defined as 0.01 absorbance change per 100 min.

The appearance of the product was measured at 290 nm using a Hitachi 124-900 double beam grating spectrophotometer. Data were plotted by means of a Hitachi 165 recorder. Protein was measured by use of biuret reagents.

Results

The partially purified enzyme exhibits total loss of activity in the absence of $MgCl_2$. Only upon the addition of Mg^{++}does this activity appear. The combination of 3.1 umoles ml^{-1} of EDTA and $2x10^{-3}$ M $MgCl_2$ yields an enzyme activity which is only 17% of that with Mg^{++} alone.

In Table 2 are presented the results of the light treatments. Regardless of the light regime, there is at minimum an increase in specific PAL activity of 7.63 times the fresh activity. Thus, other factors inherent in the aging process are also involved in the development of increased amounts of PAL. The application of light enhances this development.

The tissue aging in continuous white light shows the greatest increase in enzyme activity, being slightly greater than twice that found in tissue aged in complete and continuous darkness. It is apparent, however, that light need

Table 2.—*The Effect of Light Upon the Specific Activity of Phenylalanine Ammonia-Lyase in Potato Tissue Slices.*

Light Regime	Enzyme Units*	Protein (ug/ml)	Specific Activity (Enzyme Units/ 100 ug Protein)	Increase Over	
				FRESH	DARK
(Fresh)	2.4	470	0.51	0	0
Dark	22.2	570	3.89	7.63	1.00
Red	44.0	570	7.72	15.14	1.98
Far-Red	35.7	470	7.60	14.90	1.95
Blue	40.0	510	7.84	15.37	2.01
Light	31.8	400	7.95	15.59	2.04

*An increase in absorbance at 290 nm of 0.01/100 min = 1 unit of enzyme activity.

not be continuous to obtain this enhancement. Brief doses of red, far-red, or blue light given early in the aging process are also sufficient to cause a doubling of enzyme activity. No one broad segment of the spectrum from 380-800 nm appears to be more specific than any other. Since the action spectrum is not specific, no single pigment or chromophoric group may be implicated for PAL synthesis in potato tuber tissue slices.

Discussion

Phenylalanine ammonia-lyase plays an important role in phenolic metabolism. In dormant potato tuber tissue the amount present is neglible. Upon aging of slices, however, the amount of the enzyme increases with time until a maximum is reached around 24 hr and having chlorogenic acid as a stable end product of the pathway (Zucker, 1965).

The production of phenolic compounds and their effect upon growth and development in potato and other plant tissues is not clear. It is strikingly apparent, however, that the potato tuber itself is quiescent and that upon cutting and aeration of tissue slices the metabolic activity is increased. Two important aspects of this development involve increased respiratory rates and promotion of protein synthesis (Laties, 1962; Laties, 1963; Laties, 1967).

Stimulation of cellular activity, rather than inhibition, appears to be occurring within aging potato tissue slices. Respiration is stimulated by exogenous application of low concentrations of IAA and kinetin. Endogenous gibberellins may also play an important role in the expression of these activities (Melinder, unpublished data). Classically, the phenolic compounds produced or present within plant tissue are believed to cause inhibitory effects. It is difficult, however, to conceive of a 7-15 fold increase of PAL enzyme and concomitantly having a 2-5 fold increase in respiration during aging if this enzyme influences the production of phenolic compounds which would produce deleterious effects.

The possibility may exist whereby the inhibitors produced, such as coumarin and chlorogenic acid, may be at such low concentrations during the initial stages of aging (0-24 hr) that they could act as promoting substances (Thimann and Bonner, 1949; Thimann, 1956). One possible mechanism that could be involved would be to retard the disappearance of IAA by inhibition of the IAA-oxidase system (Andreae, 1952; Henderson and Nitsch, 1962; Zenk and Müller, 1963). Conversely, trans-cinnamic acid, the initial product formed by PAL, may act as an antagonist of auxin (van Overbeek et al., 1951).

The exact nature and mechanism by which light can influence the level of PAL activity within potato slices may be as difficult to understand. This non-photosynthetic tissue responds to light by increasing protein levels (Zucker, 1963). There is, however, no concomitant effect of light or light quality upon respiration (Hanebuth, unpublished data). By 24 hr of aging in the dark the tissue slices have increased amounts of PAL. The application of continuous light for 24 hr doubles this enzymatic development (Table II). Since short exposure to light given at 1 hr of aging and followed by darkness also produces the same results, the system appears to be saturated quickly, and not reversible by the dark period.

Even though the level of PAL is increased by light, difficulty may be encountered in terming this a photomorphogenetic response. No

action spectrum is indicated and, therefore, the involvement of one particular pigment, such as phytochrome or a metallo-protein, can not be inferred.

Summary

The isolation of phenylalanine ammonia-lyase from potato tissue has been carried out. The enzyme is stable in the cold for at least one day. There appears to be an absolute requirement for Mg^{++} to obtain activity.

The enzyme appears in tissue aged in darkness but the application of light can cause this amount to double. Short doses of red, far-red, or blue light given early in the aging process are equally as effective as continuous application of white light.

Fresh tissue of the tuber contains only small amounts of the enzyme. Tissue discs physiologically aged are stimulated to produce the enzyme in amounts 7-15 fold greater than fresh tissue.

Acknowledgments

The author wishes to thank Mr. Mark Jensen for his technical assistance. This research supported, in part, by a grant from Beta Lambda Chapter of Phi Sigma Society.

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Manuscript received July 21, 1971.

EFFECT OF THYMUS EXTRACT UPON ELECTROMYOGRAPHIC RESPONSES OF SKELETAL MUSCLE IN THE LIVING FROG

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ABSTRACT.—Electromyographic responses of the gastrocnemius muscle to injection of acetone-soluble extract of thymus glands extracted from calves were recorded on the intact living frog with bipolar electrodes. Eleven of 13 frogs injected with the extract showed a significant, although transient depression in their electromyographic responses. The thymus secretes some substance that depresses the activity of skeletal muscles. These responses, which help assay muscle activity, indicate the influence of the thymus upon muscle contraction and may ultimately be of practical importance in conditions such as myasthenia gravis.

Various attempts have been made to demonstrate the relation of thymus gland activity to skeletal muscle function. Thus, Adler (1938) induced a myasthenic reaction in the dog by injecting calf thymus extracts. Zacks and Cohen (1955) observed a decrease in muscle tension in the isolated frog sartorius muscle after injecting thymus extracts from myasthenia gravis patients. Wilson and Wilson (1955) produced paralysis of limb muscles in the mouse by injecting acetone extracts from fetal whale thymus. Nowell *et. al.* (1959) found that fetal whale thymus extracts depressed contraction of the isolated frog rectus muscle. Parkes and McKinna (1966) described a depression of contraction and of the action potentials of the isolated rat tibialis anterior muscle after injecting acetone extracts of thymus from myasthenia gravis patients. The present investigation examined the effects of injecting extracts of calf thymus on the electromyographic response (EMG) of the gastrocnemius muscle in the living intact frog.

METHODS AND MATERIAL

Electromyographic Technic

Frogs were anesthetized by intraperitoneal injection of 5.8 ± 1.2 mg/kg of sodium pentobarbital. The intact sciatic nerve of the continually moistened frog was stimulated tetanically with square-wave pulses of 2 msec duration from a Physiograph stimulator. A resistance of 800 ohms was placed in series with the electrodes so that the current remained relatively constant. The EMG was recorded from the gastrocnemius muscle and its tendon with a Hewlett Packard 141A storage oscilloscope with a 1422A time base and a 1401A amplifier.

Stimuli of increasing voltage (0.6 to 3 volts) were given at intervals of 5 minutes with about 20 to 30 stimuli until a maximal response resulted. The signal was stored on the oscilloscope and measurements were made of the first, tenth, and twentieth EMGs.

Method of Extraction

Thymus from calves was emulsified in a 1:5 w/v dilution in double distilled acetone, at 0°C (see Zacks and Cohen, 1955). All glassware used was packed with dry ice. The total volume of the filtrate was about 1 liter for every 200 gm of frozen thymus.

Liter aliquots of the filtrate were evaporated in vacuo at 37°C until the volume was 100-200 ml. The extract was transferred to a retort and acetone washings of the distillation flask were added. Continued evaporation at temperatures up to 47°C yielded a residue and vis-

cous fluid. The active thymus fraction was present in very small amounts. The 642 gm (frozen weight) of thymus yielded only 4 gm of residue and viscous fluid. The residue and fluid in 0.6% Ringer's solution will be called the acetone-soluble extract (ASE).

Biological Tests

Individual readings were taken and the values for all frogs averaged to obtain the untreated normal EMG. Additional sham controls were injected with 1 ml of Ringer's solution to find if the solvent had any effect on th EMG. Then, 13 untreated controls were given dorsal lymph sac injections of 1 ml of ASE and an average of 10 readings were obtained for each injected frog. The time during which the response to ASE was fully expressed is shown within a circle in Fig. 1.

Results

The difference in EMG between untreated frogs and frogs injected with Ringer's solution was not significant ($P > .01$).

There was no change from tetanic stimulation following solvent injection for periods up to 7 hours (Fig. 1). Thus, any weakening of response in the ASE treated frogs is not due to electrical stimulation.

The mean EMG of 13 untreated frogs was 4.57 mV. This value was significantly decreased to 2.30 mV ($P < .001$) after the injection of ASE. The average percentage drop in mV in the muscles was 52%.

With the exception of 2 frogs, Fig. 1 characterizes the response of ASE-injected frogs. The EMGs of 2 atypical frogs did not significantly change.

One frog of the 13 treated displayed an almost immediate response to ASE, a drop from 3.3 to 1.2 mV within 2 minutes after injection. The range of response in all other frogs was between 17 and 45 minutes, the period of maximum response being 20 to 40 minutes.

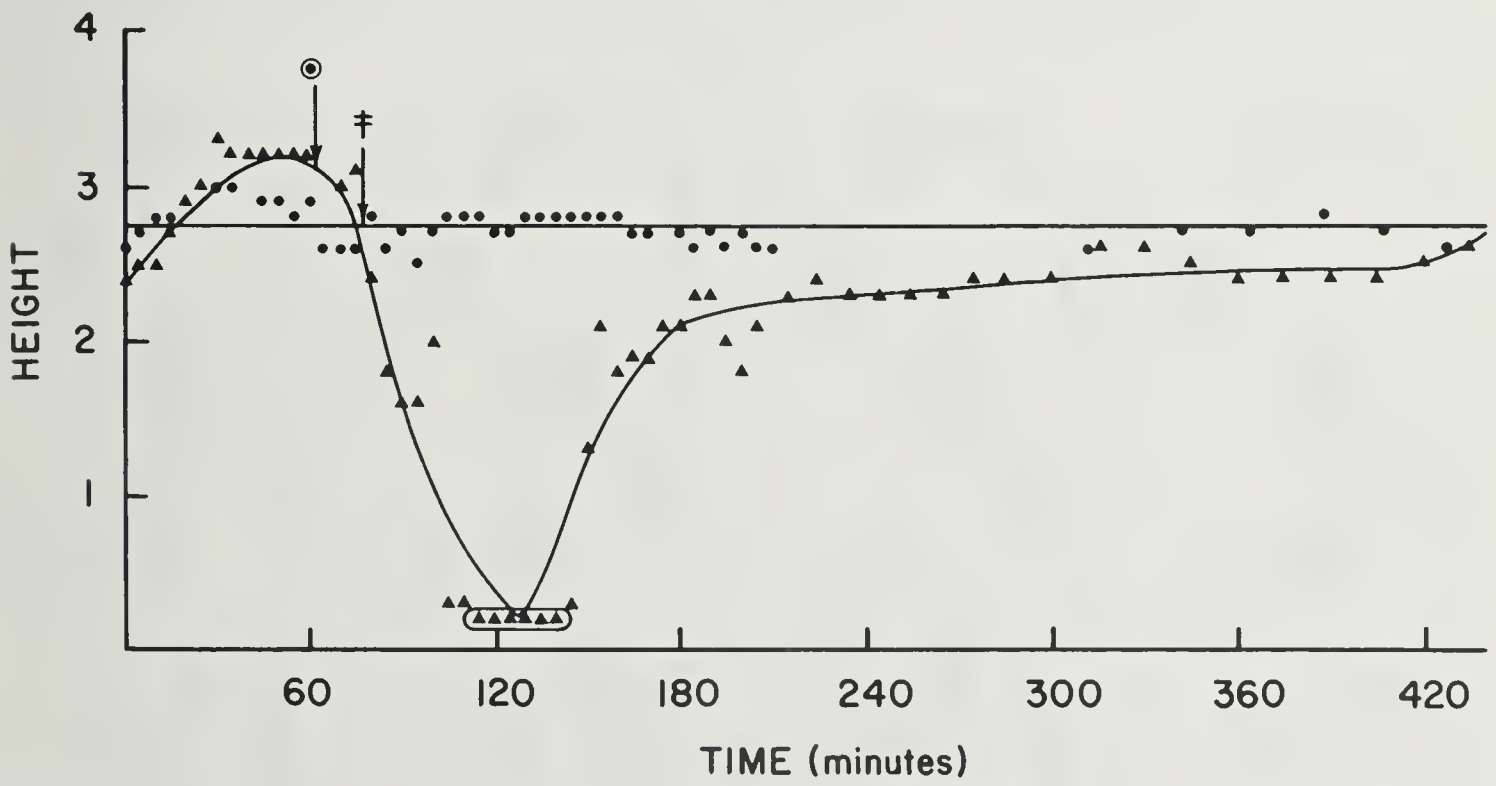

Figure 1.—Electromyographic response of a frog to thymus extract.
• = Frog injected with 0.6% Ringer's solution
△ = ASE injected frog
Arrows show time of injection; ‡ = Ringer's solution; ⊙ = ASE
Height = Electromyographic response in millivolts

Recovery, as seen in the EMG, then progressed to normal (Fig. 1).

Discussion

The significant transient depression in the EMG observed in living frogs following injections of acetone extracts from calf thymus correlates well with the findings of prior investigators (see Introduction). It is becoming increasingly evident that the thymus gland influences muscle contraction. The relationship has especial applicability to the understanding of myasthenia gravis.

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Manuscript received July 8, 1971.

A NEW SPECIES OF *HALIPLUS* FROM ILLINOIS AND SOUTH DAKOTA (COLEOPTERA: HALIPLIDAE)

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ABSTRACT.— *Haliplus tortilipenis* is described on the basis of several specimens from northern and east central Illinois, and one from South Dakota. A key is presented to distinguish *tortilipenis* from three similarly colored species, especially *connexus* Matheson with which it has been confused. A new term, the *digitus*, is proposed for a structure in the male genitalia of *tortilipenis* and other species in the genus *Haliplus*.

In the course of a survey of aquatic Coleoptera in the vicinity of Niles, Illinois, in Cook County, Mr. Sam Barakat collected an undescribed species of *Haliplus* in a clay pond in Maryhill Cemetery. Additional specimens were collected in a strip mine pond at Kickapoo State Park, Vermilion County, Illinois. It belongs to the subgenus *Liaphlus* as defined by Wallis (1933) by the presence of a striole on the upper surface near the inner margin of the posterior tibia.

Although we take full responsibility for distinguishing this species from all known Nearctic species of *Haliplus*, we are grateful to Dr. Frank N. Young, Indiana University, Bloomington, a specialist in Haliplidae, for examining the illustrations of the male genitalia and concurring with us that the species probably is undescribed.

We have introduced a new term, the *digitus*, (fig. 2B) to describe a small, jointed, finger-like lobe at the apex of the left paramere of the male genitalia of the new species herein described. It is present in many species of *Haliplus* illustrated by Wallis (1933), but appears to be absent in others. There seems to be no exact counterpart of this structure in *Peltodytes* as illustrated for several species by Young (1961). The apex of the basal part of the left paramere overlaps the base of the digitus, not clearly indicated in any of the illustrations of Wallis.

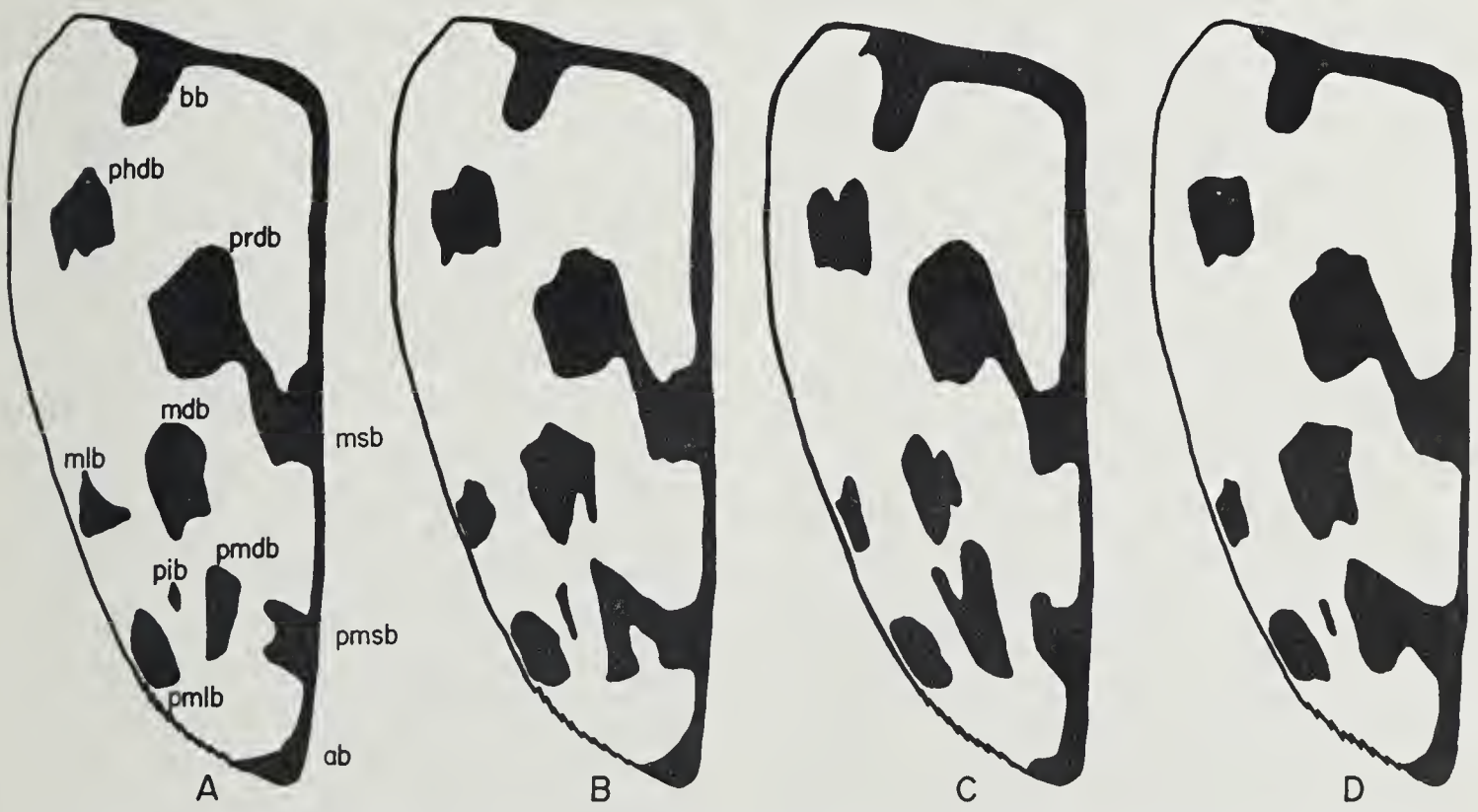

FIGURE 1.—Elytral patterns of *Haliplus tortilipenis*. A. typical; B,C,D. variations.

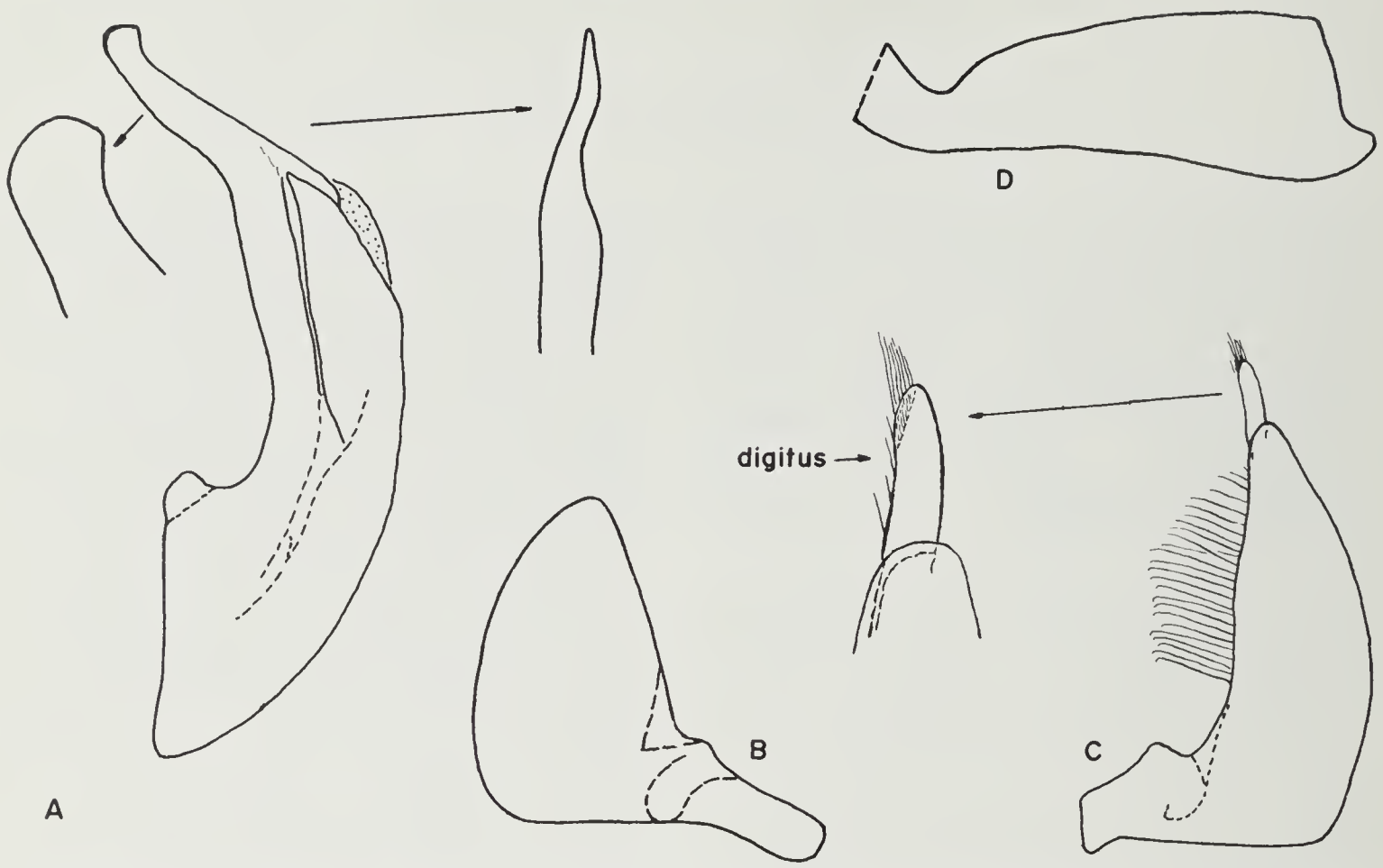

FIGURE 2.—Male genitalia of *Haliplus tortilipenis*: A. Aedeagus; B. Left paramere; C. Right paramere. D. First segment of middle tarsus of male.

Haliplus (*Liaphlus*) *tortilipenis*
new species

DIAGNOSIS.—A large *Haliplus* with distinctly maculate elytra, uniformly pale pronotum, undarkened strial punctures, slightly constricted prosternal ridge, and beaded anterior pronotal margin. Keys to *connexus* Matheson in Matheson (1912) and Wallis (1933). Superficially similar to *connexus*, *fasciatus* Aube, also *pseudofasciatus* Wallis in the subgenus *Paraliaphlus*. From all these it differs in the distinctive type of aedeagus of the male as well as in minor differences in elytral pattern from *fasciatus* and *pseudofasciatus*. The apex of the aedeagus is narrowed and curved dorsad, a unique feature among the known Nearctic species of *Haliplus* illustrated by Wallis (1933), and subsequently by Mank (1940) and Leech (1948). The aedeagus also differs strikingly from that of 15 British species of *Haliplus* illustrated by Balfour-Browne (1940, figs. 30, 31).

HOLOTYPE MALE.—Ovate, widest at or near the posterior margin of the posthumeral discal blotch. Greatest width 2.1 mm.; greatest length 3.78 mm; width of pronotum at apex .94 mm; width of pronotum at base 1.41 mm; length of pronotum along mid-line .75 mm.

Head. Front subequal in width to diameter of eye viewed from above; punctures uniformly distributed on front and vertex except for a small impunctate area between posterior margins of eyes, punctures separated by slightly less than their own widths; punctures on clypeus finer; clypeus slightly darker than labrum, the latter with a small, median, elongate dark mark; narrow dark edge of eyes conspicuous.

Pronotum. Posterior angle slightly exposed; discal punctation coarse behind the transverse impunctate area, those anterior to the impunctate area slightly coarser than those

on vertex; color nearly uniformly pale yellow, lateral margin slightly darker.

Elytra. Lateral margin broadly curved from humerus, slightly serrate in basal one-half, serrations fading behind middle to the obliquely truncate apex, truncation more finely serrate than lateral margin; strial punctures undarkened, pit-like, the rows uninterrupted; a row of fine punctures between the strial rows, these sometimes irregularly spaced; basal portion of conjoined elytra and adjacent pronotal area very slightly depressed; elytral black markings very distinct against a yellowish background, distributed as follows: (1) Suture narrowly bordered with black which does not reach first stria but reaching subsutural row of punctures; (2) postmedial discal blotch (pmdb) about 3 times longer than greatest width at anterior end, disconnected from postmedial sutural blotch (pmsb) and postintermedial blotch (pib); (3) sutural stripe expanded at apex of elytron to form a small triangle, the apical blotch (ab); (4) base of elytron narrowly black to 3rd stria, expanded into a basal blotch (bb) which covers base of 4th and 5th striae. (5) premedial discal blotch (prdb) irregular, extending from 2nd through 4th stria and connected to medial sutural blotch (msb) by a narrow strip. (6) Posthumeral discal blotch (phdb) irregular, slightly longer than wide, extending from 5th to 7th striae. (7) Medial discal blotch (mdb) with smaller lobe on inner margin, blotch extending from 4th to 6th stria. (8) Small, irregular medial lateral blotch (mlb) and (9) a larger more rounded postmedial lateral blotch (pmlb). All blotches distinctly although narrowly separated except premedial discal and medial sutural blotches.

Venter irregularly brownish yellow, legs similarly colored except for narrowly darker joints and narrow femoral base; prosternal process obscurely margined, more obviously so anteriorly, coarsely, irregularly punctured; anterior margin distinct, slightly narrower than posterior margin; sides of prosternal process constricted, width across constriction about 3/4 width of process at posterior margin; mid-metasternum in same plane, not depressed, punctures of mixed sizes, irregular; side margin of mid-metasternum distinct although undulating, extending posteriorly about 3/4 median length of metasternum; antecoxal piece with moderately large punctures; hind coxa nearly evenly and moderately closely punctured; last sternite strongly punctured on a broad lateral margin, impunctate in median triangular area; basal segment of fore and middle tarsus (fig. 2D) enlarged toward apex, the inner apical angle somewhat produced, rounded, and densely set with specialized setae; hind femur with a striole of 7 setae on inner face near apex; striole on posterior tibia close to inner fringe of marginal setae, located on apical one-half of tibia, shortened near apex, and consisting of about 7 setae.

GENITALIA.—Aedeagus (fig. 2A) about 5 times longer than wide near inner marginal concavity, slender beyond dorsal lobe to apex which is recurved toward outer margin of aedeagus; apex of aedeagus asymmetrical in caudal view (fig. 2A); left paramere (fig. 2B) slightly convex on inner margin, with long setae, some of which are angulate at tips; digitus (fig. 2B) about 3 times longer than wide, with a dense tuft of setae at the oblique apex; right paramere (fig. 2C) nearly straight on inner margin, apex roundedly angulate, inner surface

with a few scattered setae near angulation.

ALLOTYPE FEMALE.—Similar to type male in color pattern; basal segment of anterior and middle tarsus not produced as in male; size of female almost exactly that of male.

TYPES.—Holotype male: *Illinois:* Niles, Cook County, Maryhill Cemetery, August 23, 1970, Sam Barakat, in Illinois Natural History Survey (INHS). *Allotype female:* same data (INHS). Paratypes: (6♂, 8♀), same locality and collector as holotype, collecting dates as follows: August 15, 1970 (1♀); August 16, 1970 (1♂, 2♀); August 23, 1970 (2♀); August 24, 1970 (1♂); September 6, 1970 (1♀); September 7, 1970 (1♀); September 8, 1970 (2♂); September 10, 1970 (1♂); September 13, 1970 (1♂); March 21, 1971 (1♀). Paratypes from other Illinois localities as follows: Kickapoo State Park, Vermilion County, strip mine pond, August 3, 1971, Brigham and Sanderson, (1♂, 1♀); Illinois (no other data) (1♂); Dead River, Dunes Park near Waukegan (Lake County), April 19, 1947, H. S. Dybas (1♂, FMNH). *South Dakota:* Brookings (Brookings County), September 11, 1939, H. C. Severin, gravel pit (♂1, C. U.). Paratypes in collection of Illinois Natural History Survey, W. U. Brigham, S. F. Barakat, Cornell University, Field Museum of Natural History, F. N. Young, U. S. National Museum, California Academy of Sciences, G. W. Folkerts.

VARIATION.—Gross variation within the type-series of *Haliplus tortilipenis* is limited to the elytral maculation. Location and intensity of the blotches appears to be constant, with variation apparent only in the shape of the blotches, the tendency toward coalescence of the blotches, and the width of the sutural stripe posteriorly. The male genitalia showed insignificant variation in all individuals of this species. Male and female paratypes showed no important differences in length and width. They ranged in length from 3.6 mm. to nearly 4 mm, average of 12 individuals 3.7 mm; and in width from 2.0 mm to 2.2 mm, average of 12 individuals 2.1 mm.

Little significance is attached to the shape of the elytral blotches. Variation of the other characters is presented in fig 1. The holotype, allotype, and seven paratypes show the most frequently occurring set of characters (fig. 1A). This condition is defined in the diagnosis of the holotype. Coalescence of the postmedial discal blotch (pmdb) and the postmedial sutural blotch (pmsb) is shown in six paratypes (fig. 1B). Two paratypes show coalescence of the postintermedial blotch (pib) and the postmedial discal blotch and the "wide" (reaching first elytral stria) condition of the sutural stripe between the postmedial sutural blotch (pmsb) and the apical blotch (fig. 1C). A single paratype (fig. 1D) shows coalescence of the postmedial discal blotch and the postmedial sutural blotch, and the wide condition of the sutural stripe between the postmedial sutural blotch and the apical blotch.

DISCUSSION.—This species is closely similar in elytral blotch pattern to some individuals of *connexus* Matheson, and we were unable to find consistent differences to distinguish all individuals of the two species except in the male genitalia. The differences here are striking, suggesting the isolation of *tortilipenis*, for *connexus* has a type of genitalia much more similar to other species in the genus. *H. connexus* averages smaller than *tortilipenis*, and in the type-series of *connexus* there is an average greater degree of

coalescence among elytral blotches. The two species are assumed to be sympatric at Dunes Park in Lake County, Illinois, based on one record of a male of *tortilipenis* and three males of *connexus*, the latter evidently collected earlier when Dunes Park then was known as Beach. Young (1961) discusses superficial resemblance of sympatric species in the genus *Peltodytes*. He suggested that the bright elytral patterns might be aposomatic and that mimicry could be involved. No such convergence of color pattern was apparent among the other species of haliplids occurring with *tortilipenis*. This does not rule out Young's observations of apparent mimicry for most haliplids are somewhat similar in appearance and the degree of resemblance might be sufficient for effective Mullerian mimicry.

ECOLOGY.—All of the 1970 collections of *Haliplus tortilipenis* were taken from a cemetary pond in Niles, Cook County, Illinois. Data supplied by the collector, Mr. Sam Barakat, provide the basis for the following discussion.

The area of the pond is approximately 0.4 ha. and it has a clay bottom. Maximum depth is about 1 m. The center of the pond is dominated by emergent vegetation (*Scirpus* and *Typha*). Sedges are predominant along the periphery of the pond. Much of the pond supports dense growths of filamentous algae. The pond occasionally dries up.

A very diverse water beetle fauna exists in the pond, 41 species having been taken during 1970. Nearly 500 haliplids were taken during the period April through October, 1970, but only 15 of these were *Haliplus tortilipenis*. *Haliplus triopsis* Say was the predominant haliplid. *Haliplus immaculicollis* Harris and *Peltodytes edentulus* (LeConte) were present in lesser numbers. A few individuals of *H. subguttatus* LeConte and *H. borealis* LeConte were also taken.

The apparent scarcity of *H. tortilipenis* may stem from one of two possibilities: 1) It may exist in extremely low numbers, or 2) it may be confined to the deeper portions of the pond where collections are taken less frequently and with more difficulty.

Haliplus (*Liaphlus*) *connexus* Matheson

Because of the close similarity of *tortilipenis* and *connexus*, we suspected that both species might be found in collections identified as *connexus*. Through the courtesy of Dr. L. L. Pechuman at Cornell University, we were permitted to examine the entire series of *connexus* including the types. All of the specimens, except one of *tortilipenis* from Brookings, South Dakota, proved to be *connexus* as we understand it. Matheson did not designate types in his description nor did he give the type-locality. However, a holotype, allotype, and paratypes were labeled by him. We take this opportunity to clarify these points, also to record all paratypes and give additional distribution records represented in the Cornell series.

HOLOTYPE ♂, and allotype ♀, Ithaca, *New York*, May 10, 1910. Paratypes (38♂, 39♀) from Ithaca, New York as follows: June (1♀); August 4, 1885, J. M. Stedman, L. J. Martin, (1♂, 4♀); September 19, 1909, (15♂, 12♀); October, 1909, (5♂, 3♀); October 2, 1909, Inlet Valley (2♂, 3♀); October 11, 1909, Cascadillo Creek (6♂, 7♀); October 15, 1909, (2♀); October 24, 1909, (1♂, 1♀); October 25, 1909, Fall Creek (5♂, 2♀); November 1, 1909, (1♂, 1♀); June 1910, (2♂, 3♀). Paratypes from other localities mentioned in

the description as follows: *Nova Scotia*, (Truro) August 30, 1902, R. Matheson (1♂, 2♀); September 1, 1909, R. Matheson (1♂, 2♀). *Massachusetts*, Amherst, July, 1910 (1♂). *Michigan*, Lansing, (labeled Agricultural College), October 14, 1910, (1♂), October 31, 1910, (1♀). *Illinois*, Lake Forest, (1♀). Other localities represented in the Cornell series of *connexus* are: *Connecticut*, Cornwall, October 30, 1918 (1♂, 2♀); April 12, 1920, (1♀); May 16, 1920 (1♂, 1♀); November 8, 1920, (2♂, 1♀); July 1, 1939, (1♀), all collected by K. F. Chamberlain. *Massachusetts*, Framingham, April 8, 1923, C. A. Frost, (1♂); Sherborn, April 6, 1924, C. A. Frost, (1♂). *New Hampshire*, Claremont, June, 1908, (1♂).

In addition to the foregoing localities for *connexus*, Wallis (1933, p. 45) added Minnesota. Gordon and Post (1965, p. 8) added a record for North Dakota, and the illustration given for the male genitalia appears to be that of *connexus*.

Twenty-seven male and female paratypes from Ithaca, New York ranged in length from 3.3 mm to 3.6 mm (average 3.4 mm), and in width from 1.6 mm to 1.9 mm (average 1.8 mm). Females from Lansing Michigan and Toronto, Ontario were slightly wider, and ten specimens from Cornwall, Connecticut averaged slightly longer than the Ithaca specimens (3.56 mm) and wider (2.0 mm). These averages are a little less than those of the small numbers of *tortilipenis* studied.

Key to Distinguish *Tortilipenis* from Similarly Colored Species

Males

1. Sutural black stripe reaching first stria anterior to medial sutural blotch; some strial punctures in basal half of elytra may be darkened; elytral apex at most faintly undulate..... .....*fasciatus* Aube, *pseudofasciatus* Wallis

   Sutural black stripe not reaching first stria anterior to medial sutural blotch; strial punctures never darkened; elytral apex distinctly serrate-denticulate.....2

2. Aedeagus short and blunt (Wallis, 1933, fig. 23d), apex evenly curved toward inner margin; length of Ithaca, New York specimens from 3.3 mm to 3.6 mm, average of 27 individuals 3.4 mm ....*connexus* Matheson

   Aedeagus (fig.2A) slender toward apex, apex somewhat abruptly curved toward outer margin; length from 3.6 mm to 4 mm, average of 12 individuals 3.7 mm.....*tortilipenis* new species

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Manuscript received May 13, 1971.

A MORPHOLOGICAL STUDY OF MASTOPHORUS NUMIDICA (SEURAT, 1914) READ AND MILLEMANN, 1953 (NEMATODA: SPIRURIDAE)

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ABSTRACT.—Morphological studies on *Mastophorus numidica* were undertaken in an attempt to evaluate the reliability of various characters used for taxonomic purposes by determining the extent of their morphological variation. The results of this study and a study of the data presented by previous authors showed that the presence or absence of pseudolabial teeth, the shape of the stoma and the form of the spicules are the most reliable characters for separating species of the genus *Mastophorus*.

The use of certain morphological characters deemed reliable by some workers proved uncertain for differentiating *Mastophorus numidica* (Seurat, 1914). A review of the literature revealed that there has been much controversy concerning the significance of taxonomic criteria in separating species of this genus. The variability of these characters was studied in several specimens of *M. numidica* in an attempt to evaluate their reliability.

METHODS AND MATERIALS

Specimens of *M. numidica* were collected from the stomachs of deer mice (*Peromyscus maniculatus*), cleansed in physiological saline, killed and fixed in 70 per cent ethanol and 5 per cent glycerine, cleared, dehydrated and mounted in pure glycerine. The teeth were studied in profile according to the method of Hsu (1935) by cutting off the head and splitting it longitudinally. Posterior ends of males were cut, straightened and flattened in order to study the spicules and papillae. All measurements are given in millimeters with the mean in parenthesis.

DESCRIPTION

Based on 27 males and 32 females. Mouth surrounded by two trilobed lips, median lobe larger than lateral ones; number and shape of teeth vary, usually four distinct or two indistinctly divided teeth on each lobe; each lip with two cephalic papillae, stoma laterally compressed. Cervical papillae anterior to excretory pore, position of papillae in relation to nerve ring varies widely. Esophagus long, differentiated into two regions.

Male: Length, 11.00 to 22.12 (17.02); maximum width, 0.36 to 0.61 (0.46); vestibule and esophagus 0.09 to 0.15 (0.12) and 3.10 to 6.30 (4.70) long, respectively; distance from anterior end to excretory pore, 0.30 to 0.44 (0.39). In fixed specimens, posterior end of body curved ventrally forming one or two loops. Length of tail, measured from anus to posterior extremity, 0.18 to 0.40 (0.26); caudal alae well developed and equal; ventral surface ornamented with cuticular elevations; cloacal apeture bordered by two lips that project slightly; caudal papillae stalked, but stalks covered with outer cuticle so that in surface view only rounded ends of papillae visible, four pairs of large precloacal papillae close to median line, a single large papillae located immediately in front of the anterior border of the cloaca; two pairs of large post-cloacal papillae followed usually by four to six smaller papillae. Spicules unequal; right spicule 0.78 to 1.18 (0.90) long, wing like and

transversely striated; left spicule 0.28 to 0.42 (0.33) long, filaform.

Female: Length, 11.22 to 36.08 (26.09); maximum width, 0.64 to 1.22 (0.91); vestibule and esophagus 0.12 to 0.30 (0.16) and 3.10 to 6.30 (4.70) long, respectively; distance from anterior end to excretory pore 0.34 to 0.53 (0.43). Vulva posterior to middle of body, its position expressed as percentage of body length being 51 to 66 (56) per cent; vagina tubulous and lined with glands arranged in moniliform series; ovijector heavily muscularized; distance from anus to posterior extremity, 0.18 to 0.38 (0.27). Eggs, 0.054 to 0.059 (0.057) by 0.043 to 0.046 (0.045), ovoid and thick shelled, embryonated at time of deposition.

Results and Discussion

Seurat (1914) proposed the genus *Protospirura* with *P. numidica* as type. According to Seurat (1916), *M. echiurus* Diesing, 1853 is the larva of *P. muris* (Gmelin, 1790) and he synonymized *M. echiurus* with *P. muris*. Since *M. echiurus* was designated as the type species of *Mastophorus*, *Protospirura* should have been invalidated.

Chitwood (1938) proposed the retention of both genera, inasmuch as all species reported up to that period could be classified under one genus or the other.

Subsequent to the appearance of Chitwood's publication several new species have been reported which possess characters of both *Protospirura* and *Mastophorus* (of Chitwood) and some species with characters which differ from both genera.

Rather than erect new genera, Read and Millemann (1953) supressed *Protospirura* as a synonym of *Mastophorus*. Considered as valid specific characters were the presence or absence of teeth on the lips, the shape of the stoma, the length of the male tail, the position of the vulva and the stalked or sessile nature of the papillae.

In the present study, the number of teeth, the absolute length of the spicules, the number of genital papillae and body measurements were found to vary greatly in the specimens of *M. numidica* examined. Similar findings were reported for other species of *Mastophorus* by Chitwood (1938), Read and Millemann (1953) and Wertheim (1962). The position of the vulva and the length of the male tail were also found highly variable in the material studied.

Analysis of the present data and that presented by previous investigators substantiates the conclusions of Wertheim (1962) that the presence or absence of teeth on the lips, the shape of the stoma and the form of the spicules are the most reliable characters for separation of species of *Mastrophorus*.

Summary

A study of the variability of morphological characters in specimens of *M. numidica* has shown that the most reliable characters for species differentiation are the shape of the stoma, the presence or absence of teeth on the lips and the form of the spicules.

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Manuscript received August 23, 1971.

AN ANALYSIS OF MORPHOLOGICAL VARIATION: A COMPARISON OF SOME STREAM-DWELLING FISHES

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ABSTRACT.—Nine species of fish from two different streams are compared with respect to variation of some non-meristic characters. It appears that pelagic species are more variable than benthic ones and that differences in morphological variation may reflect niche size differences.

INTRODUCTION

Most studies of morphological variation in fishes have considered only meristic characters and many of these have dealt solely with geographic variation (Bryan, 1969; Olund and Cross, 1961). Meristic character variation is often the result of developmental inconsistencies rather than direct adaptive mechanisms (Albaugh, 1969). The present study concerns itself with variation of non-meristic characters among a number of different species. Meristic characters were shown to be almost completely invariable in these fishes in a preliminary study by the authors.

The amount of variation of any morphologic feature or complex of features is related to the amount of selection (or lack of) for precision in that character. The degree of centripetal, or stabilizing, selection determines the degree of variation allowable (Bader and Hall, 1960). Some phenotypic characters may exhibit only narrow ranges of variation, as greater deviations would prove detrimental and thus be selected against. The characters that are permitted some degree of variability and the relative degree of their variability are easily determined. Why only some characters are more variable than others in one species and why the degree of variation of the characters differ between species is another matter.

Thus we attempt here to investigate the relative amounts of morphologic variation in nine species of stream-dwelling fish. The species were divided into two groups according to their feeding habits: mid- or top-water feeders, and bottom feeders. The intent is primarily to determine, by comparing degrees of morphological variation, whether greater selection pressures are placed upon pelagic or benthic feeders.

METHODS AND MATERIALS

The fish were collected by seining from two different and widely separated streams of the Salt Fork and Kaskaskia drainage systems which we will call streams 1 and 2, respectively. Nine species were collected from stream 1 and seven from stream 2 (Table 1). Collections were made in the fall of 1969 and 1970. After eliminating the species caught in small numbers, nine species remained for investigation (Table 1). Ninety-six percent of the total number of fish and 67% of the total species caught in stream 1 were utilized; 95% of the number and 71% of the species from stream 2 were studied. A Mann-Whitney U-test (Siegel, 1968) performed on the coefficients of variation of the nine species from the two streams indicated that there was no significant difference in variation between the two groups; hence the fish were considered as coming from a single population.

TABLE 1

Species	Collected in Stream No.	N	Feeding Location
Fundulus notatus	2	34	Pelagic
Semotilus atromaculatus	1 & 2	81	Pelagic
Notropis lutrensis	2	36	Pelagic
Notemigonus chrysoleucas	1	30	Pelagic
Notropis dorsalis	2	26	Benthic
Pimephales notatus	1	108	Benthic
Ericymba buccata	1	57	Benthic
Campostoma anomalum	1 & 2	61	Benthic
Cyprinus carpio	1	24	Benthic
Notropis spilopterus	1	5*	
Notropis stramineus	1	6*	
Etheostoma blennoides	1	1*	
Hybognathus nuchalis	2	8*	
Phenacobius mirabilis	2	1*	

*Not considered in study due to small sample size

Using dividers, ten characters were measured on each fish. Measurements were made according to Hubbs and Lagler (1958). The characters, measured in mm., were: length of dorsal fin base, length of anal fin base, length of longest pectoral ray, length of longest pelvic ray, head length (anterior portion of snout to posterior part of operculum), head width (greatest dimension of head when jaws and operculae are closed (and operculae lie flat), orbital width, upper jaw length, snout length (anterior point on upper lip to anterior part of orbit), and standard body length.

The measurements of the body parts were expressed as proportions of the standard length since body size varied between individuals. In all species the size range was less than 20 mm, thereby minimizing the effects of allometric growth.

The physical structure of both streams is quite similar, averaging 10-15 feet wide, 2-3 feet deep, and having a gravel bottom covered with organic silt. The stream gradient is low (3 to 4 feet per mile) and the water flow is moderate to sluggish during normal water stages; riffles are gentle and pools generally shallow. Water levels change rapidly and drastically and flooding occurs regularly, especially in the spring and summer, during which the water may rise substantially and erode the stream banks considerably. Water temperatures fluctuate a good deal (as much as 8 degrees C in the summer) and in the winter the streams may freeze to the bottom, forcing fish into deep pools. The water turbidity is usually high, drastically limiting the growth of aquatic vegetation. The two drainages are relatively unpolluted and have a slightly alkaline pH. (See Larimore and Smith, 1963, for further details on the drainages of Champaign County, Illinois).

Fish Species Examined

A brief description of each of the nine species studied follows, with the major emphasis on the differences in habitat and food.

Of the five species in the study which can be classified as benthic or primarily bottom dwelling, the carp, *Cyprinis carpio*, alone seems to prefer warm, low gradient streams with an abundance of organic matter. Its tolerance, however, extends over a wide range of bottom types and turbidity levels. Organic detritus and plant matter

are the major constituents of the carp's diet (Mauck, *et al.*, in press).

The bluntnose minnow, *Pimephales notatus*, exhibits the highest degree of tolerance of any of the species. It has the ability to survive and spawn in virtually all types of waters, in gradients ranging from 0-100 feet, in areas of high turbidity and pollution, and under conditions of severe competition. Primarily herbivorous, the bluntnose obtains its food by scraping algae from the substrate (Stegman, 1959).

A widespread species, the stoneroller minnow, *Campostoma anomalum*, is closely associated with riffles, especially in low gradient streams. With its ventrally positioned sucker-like mouth, it is particularly adapted to feeding on diatoms, filamentous green algae and other small plants adhering to the riffles' stony substrate (Kraatz, 1923).

The last two benthic minnows, the silverjaw, *Ericymba buccata*, and the bigmouth shiner, *Notropis dorsalis*, closely resemble each other morphologically and in their habits. They possess a similarly shaped head, flattened on its ventral surface, have similar body proportions and coloration, and possess large horizontal mouths. The large mucous ducts visible at the bottom of the silverjaw's head readily distinguishes the two species. Both occur most frequently in gravel- or sandy-bottomed stream sections of moderate gradients, and exhibit similar schooling, feeding, resting and retreat behavior (Hoyt, 1970; Trautman, 1957). Hoyt (1970) states that the silverjaw forms a close feeding association with the bluntnose and stoneroller. In scraping off patches of algae covering the substrate, these two herbivores expose larval insects which constitute the majority of the silverjaw's diet. No food data are available for the bigmouth shiner.

Of the pelagic species, the golden shiner, *Notemigonus chrysoleucas*, appears most frequently in sluggish streams with bottoms chiefly of organic debris and/or sand, substantially covered by vegetation. It utilizes a wide range of food resources which includes mollusks, insects, and a variety of plants (Forbes, 1908).

The northern creek chub, *Semotilus atromaculatus*, is a ubiquitous species which occurs in greatest densities in small streams of medium to high gradients, especially above riffle areas. Mayfly naiads, coleopteran beetles, and plant material compose the largest portion of the chub's diet with larger adults also capturing small fish (Dinsmore, 1962).

The redfin, *Notropis lutrensis*, is an inhabitant of shallow pools of moderate current and is primarily insectivorous. Terrestrial arthropods falling or swept into the water comprise more than two-thirds of its total diet, with aquatic insect larvae and plant material making up the balance (Lewis and Gunning, 1959).

The black-striped topminnow, *Fundulus notatus*, is most frequently found in streams of low or base gradients where at least some aquatic vegetation is present. It is primarily a surface swimmer and its major food items consist of terrestrial arthropods, along with aquatic insects and filamentous algae (Thomerson and Wooldridge, 1970).

Analysis

The means, variances, variances of the logs of the measurements (base e), and coefficients of variation were calculated for each character of each species. By means of an F-test using the variance of the logs, each species was tested against

every other for each character to compare the intrinsic variations (Lewontin, 1966). The coefficients of variation were grouped in two ways and Mann-Whitney U-tests were performed on them. The species were divided into pelagic and benthic groups to see if there were any significant differences in the variability of characters between the two groups as a whole.

It appears that the comparison of character variabilities over pairs or groups of species is much more useful for our purposes than comparing the variations of all characters between individual species. This character-comparison technique gives hints as to where selection is acting and how strong it is, rather than merely demonstrating that one species is more variable than another.

Results

The results indicate that the pelagic species measured are significantly more variable ($p < .005$) than the benthic fish in three of nine characters measured (dorsal and anal fin base length, and pelvic fin (length). The benthic fish are not more variable than the pelagic fish for any characters (see Table 2).

Discussion

In general it seems that selection pressures are greater on benthic than on pelagic ones, at least for the three characters indicated. Centripetal selection appears to act with similar vigor on the other characters in both groups. An examination of each of the features studied may clarify the discrepancy in variabilities between the divisions. As shown by a Mann-Whitney U-test, the dorsal fin length is significantly ($p < .004$) more variable in the pelagic fish. The dorsal fin has the logical function of acting as a keel (Lagler, *et al.*, 1962) aiding the fish in maintaining an upright posture, but a thorough study of the function of the dorsal fin would have to be made before the differences in variability of this structure in the two groups could be explained.

Anal fin length ($p < .002$). A similar argument can be made here for the anal fin.

Pelvic fin length ($p < .002$). Selection might aim towards the maintenance of an optimum pelvic fin length in the benthic fishes rather than in the pelagic fishes because the former appear to use the pelvic fin more to maintain their position on the bottom (Allen, 1969).

We at first found the results surprising in that they are almost opposite of what we expected. A preliminary scanning of the situation hinted at a greater pressure of stabilizing selection on pelagic fish due to a seemingly more rigorous habitat. But results indicate the reverse. Although niche width is extremely hard to measure, it is probably safe to assume that the niches are wider and fluctuate more in the pelagic rather than bottom-dwelling species.

The water level, temperature, turbidity, and current velocity change rather continually. These fluctuations may affect the parameters of the pelagic niches more than the benthic ones. Thus the pelagic fish, living in a more variable environment, adapt to the environment by having more variable individuals since the chances that an individual is present which would be optimally adapted to the environment at any one time would be greater in a more variable population. A population which lives in a more stable environment should be less variable as selection pressures are temporally similar. The wider pelagic niches allow greater phenotypic variation by adaptation

TABLE 2—Coefficients of Variation for Each Species/Each Character

	Dorsal Fin	Anal Fin	Pectoral Fin	Pelvic Fin	Head Length	Head Width	Orbit	Upper Jaw	Snout
P. notatus	7.49	12.23	7.37	6.99	4.88	6.62	10.23	12.47	10.61
S. atromaculatus	12.98	11.45	10.35	20.74	4.04	8.24	11.68	12.96	10.21
N. lutrensis	17.05	12.66	10.80	13.71	5.23	13.49	13.27	16.59	12.75
N. chrysoleucas	18.42	14.24	10.39	10.26	6.53	15.01	15.34	19.75	17.12
N. dorsalis	10.90	9.87	9.39	9.55	5.58	7.99	11.63	14.10	10.90
F. notatus	9.40	12.46	9.04	18.79	5.10	9.23	12.64	9.32	12.10
E. buccata	9.59	6.86	5.61	7.50	3.97	6.55	12.86	4.79	6.39
C. anomalum	8.84	8.15	6.79	7.12	5.97	6.91	9.79	12.37	10.34
C. carpio	9.14	9.53	11.76	8.00	7.86	6.07	50.46	43.14	7.35

to the diverse factors in the environment and, as Van Valen (1965) states, morphological variation tends to be greater in populations with wider niches because the variation is adaptive.

We can apply these ideas to the differences in niche width between benthic and pelagic fish. Since the benthic situation may not vary as widely as the pelagic, the benthic environmental range is smaller, and the optimal single phenotype that exists also does not vary as much as the optimal pelagic phenotype since the latter has to be intermediate between more widely different environments. We feel that the data give stimulus to these ideas if not actually supporting them, but we make our conclusions cautiously. The species considered may not adequately represent the two feeding categories and the variation may not be determined solely by selection. Willson (1969) believes that morphological variation generally may not reflect niche size. We feel that differences in morphological variation reflect *difference* in niche size, but a lack of differences in this variation does not necessarily indicate the opposite situation.

Summary

Pelagic- and benthic-feeding fishes from two streams are compared with respect to the morphological variation of nine non-meristic characters. Pelagic species on the whole were found to be more variable, indicating weaker selection pressures on this group. It appears that the niche of the pelagic species is wider since environmental fluctuations are greater in the pelagic environment. Differences in morphological variation may reflect differences in niche size.

Acknowledgments

We would like to thank Drs. T. H. Frazzetta, R. R. Roth, F. W. Schwartz, M. F. Willson, and L. Van Valen for their helpful comments and criticisms of the manuscript although they do not necessarily agree with the finished product. L. Page provided us with information on feeding habits, D. Schemske aided us in the identification of some of the fish species collected, and B. Buckingham spent many hours retyping the manuscript. Computer time was provided by the Department of Zoology, University of Illinois, Urbana.

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Manuscript received August 2, 1971.

SUCCESSION IN AND COMPOSITION OF A CENTRAL ILLINOIS PRAIRIE GROVE

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ABSTRACT.—The composition and succession of a mature stand in Funk Forest, McLean Co., Illinois were examined by standard ecological methods during June and July of 1970. Tree density in a sample from this prairie grove is 202 trees/ha (> 10 cm dbh) with a basal area of 28.5 m^2. Succession is tending to a maple climax. Sugar maple (*Acer saccharum*) is the dominant tree species, followed by oaks and ashes. Maples and oaks are randomly distributed. A sugar maple-pawpaw (*Asimina triloba*) understory of 4890 saplings/ha has an aggregated dispersion. Pawpaw and sugar maple saplings are negatively associated. Herb density is 83 stems/m^2. The herb dominants, in order of importance, are wild ginger (*Asarum reflexum*), wood nettle (*Laportea canadensis*), and large waterleaf (*Hydrophyllum canadense*). A nettle-ginger association is common. The large waterleaf is negatively associated with ginger and most other herbs. Other analyses include coefficient of community and Simpson's diversity index.

Funk Forest is a remnant of a prairie grove that once occupied a five-square-mile area along Timber Creek in McLean Co., Illinois. These groves, a striking feature of the Illinois prairie, are isolated from main bodies of timber and found on uplands along watercourses or on rough morainal land. Most of these formerly numerous prairie groves have now been cleared for agriculture.

Several workers have studied the ecology of this unique type of woods. Gleason (1912) made the first phytogeographical study of a prairie grove. Telford (1926), Boggess (1964), and Boggess and Bailey (1964) analyzed the woody vegetation of two similar stands in central Illinois. Clark (1947), Calef (1953), and Boggess and Geis (1966) have done the only previous vegetational studies of the Funk Forest area. Clark reported tree percentages from transect data. Calef, Boggess, and Geis worked in the 60-acre University of Illinois' Funk Forest Natural Area. Calef's data on the woody vegetation was based on a strip transect survey. Boggess and Geis examined the woody vegetation of the same area twelve years later, and also presented data on soil profiles.

Prior to our work, no comprehensive ecological study had been done in Funk Forest. The primary objective was to quantitatively and qualitatively determine the floristic composition of this woodland community. A second objective was to investigate the interrelationships among the herbs, saplings, and trees. The final objective was to discover the successional trends in this prairie grove.

Funk Forest is located in McLean Co., Illinois (89° 08′ W Long; 40° 21′ N Lat). Much of the original forest area was cleared for agriculture, and presently only several hundred hectares of woods remain, much of which has been heavily grazed and selectively cut in the past. Timber Creek is the most notable topographic feature of the forest. The relief of the area varies from 195 m to 210 m above sea level. Funk Forest is more mesic than the typical oak-hickory woods of central Illinois. Our study area has flat, fairly moist, upland topography, and was selected because it is one of the few mature, undis-

turbed tracts available. Subjectively we noted that our plot has a homogenous distribution of tree species. There are drier tracts in the forest that have a higher oak frequency.

The forest is in the Till Plains Section of the Central Lowland physiographic province and is on a loess deposit covering the LeRoy moraine, which is of the Tazewell Substage of the Early Wisconsin glaciation, formed during the Late Pleistocene (Wascher *et al.*, 1960). In a study of a similar prairie grove, Vestal and Heermans (1945) suggested that the groves developed 400 to 600 years ago on uplands occupied by prairie; however, Gleason (1912) proposed that fires spreading from the adjacent prairies isolated the groves from pre-existing main bodies of timber.

The Funk Forest climate is classified as the humid continental type. Great variations in temperature and precipitation are typical. A climatological summary of the area shows that annual precipitation averages 36.7 inches (Illinois State Water Survey, 1964), annual temperature averages 11-12 C, with a mean growing season of 171 days extending from April to September (Fehrenbacher and Wascher, 1967).

A detailed soil survey of the forest has not yet been undertaken. Recent surveys of Odell (1957) and Fehrenbacher and Wascher (1967) described this soil as Grey-brown Podzolic, Alfisoil order, Birkbeck soil association M. This association typically has a silt loam A horizon, a heavy silty-clay loam B, and a glacial till C. Boggess and Geis (1966), working in a similar area of Funk Forest, found the A horizon to be 30 cm deep and encountered C at 1.5-2.0 m. Organic matter of the A horizon was 5%, and pH was 6.0.

Methods and Procedures

The field study was carried out from Mid-June through July 1970. A 1-hectare area was used as the study plot, and was subdivided into 676 quadrats. Quadrats were randomly selected when sampling from the entire hectare proved impractical.

Trees were classified into three groups based on diameter at breast height (dbh): trees, $>$ 10 cm; saplings, 2.5 to 10 cm; and seedlings, $<$ 2.5 cm. We recorded species and dbh for all trees in the hectare. Density, relative density, dominance, and relative dominance were calculated from standard formulas (McIntosh, 1957). We modified the importance value of Curtis and McIntosh (1951), as did McIntosh (1957) and Boggess (1964), to calculate the importance value index (IVI = 200). Using the same class sizes and methods as Boggess and Geis (1966), we calculated basal areas by diameter class.

Saplings were studied by transect and line interception methods. We modified the transect methods of Calef (1953) by using a 2 m transect width along 189 m of grid lines. A sapling stem count was taken at 3 m intervals, and density and frequency were calculated. We graphed number of individuals per interval against transect length to show relationships between the two dominant sapling species. The line interception method of Buell and Cantlon (1950) was used to obtain sapling dominance. The intersect lengths over a 150 m hectare diagonal were measured, and cover area calculated. The importance value (Curtis and McIntosh, 1951) was obtained by adding the relative density and frequency values computed from the transect intervals with the relative dominance value

calculated by line-intercept (IV = 300).

The frequencies and densities of herbs and seedlings were measured in 15 randomly selected 1-m^2 quadrats. Another 150 m line intercept was used to find herb dominance. We graphed meters of herb cover against intercept line length to show relationships that the quadrat method does not emphasize. Importance values were calculated by adding the relative dominance value from the intercept method to the relative density and frequency figures from the quadrat technique (IV = 300).

An intrapopulation dispersion analysis (Bray, 1956, 1962) was done on each dominant tree species, and evaluated by a Chi-square test. Sapling dispersion was calculated using the variance to mean ratio method based on the Poisson distribution (Curtis and McIntosh, 1950; Cottam, Curtis, and Hale, 1953).

Dice (1945) and McIntosh (1957) described the contingency table method for calculating interspecific association, and Cole (1949) described the Chi-square test used to determine the significance level. This method was used to verify suspected negative associations between the two dominant sapling species and among the herb dominants.

The Simpson's index of diversity (Simpson, 1949) was calculated from our tree data and also from the earlier data reported for the Funk Forest Natural Area by Boggess and Geis (1966). Likewise the community similarity coefficients (Bray and Curtis, 1957) for these areas were calculated.

Plant nomenclature is that of Jones (1963).

Results

The hectare contained a total of 202 trees (> 10 cm) with a total basal area of 28.5 m^2 (Table 1). Total sapling density was 4890 per hectare (Table 2). The total herb density was 83 stems per m^2 (Table 3).

The intrapopulation dispersion analyses done on sugar maple (*Acer saccharum*) and oak trees showed no significant differences from random distribution at the .05 level. Dispersion analyses on saplings showed they have contagious distributions: the variance to mean ratios calculated for pawpaw (*Asimina triloba*) and sugar maple saplings were 2.19 and 2.92 respectively, which were significant at the .05 level.

Transect data (Fig. 1) illustrate the negative association between pawpaw and sugar maple saplings, and show their contagious distributions. The interspecific association between pawpaw and maple saplings calculated by the contingency table method was −.295. A Chi-square test showed this to be significant at the .05 level.

The distribution of the three herb dominants, wild ginger (*Asarum reflexum*), wood nettle (*Laportea canadensis*), and large waterleaf (*Hydrophyllum canadense*), is illustrated by a graph of a portion of line intercept (Fig. 2). The intercept data also revealed that appendaged waterleaf (*H. appendiculatum*) and wild ginger often occurred under wood nettle, but rarely under large waterleaf. Most other species also occurred with wood nettle rather than large waterleaf, and oak seedlings showed no relationship with herb distribution, but were dispersed around parent oaks. Interspecific association analyses of the wild ginger-large waterleaf and wild ginger-wood nettle relationships showed the former to have a nega-

TABLE 1.—Number of Trees and Basal Area (m^2) per hectare by Diameter Class and Importance Value Index

	Diameter Classes (cm)												
	10-15		16-30		31-61		62-91		92 +		Total		
Species	N	B.A.	N	B.A.	N	B.A.	N	B.A.	N	B.A.	N	B.A.	IVI
Acer saccharum	28	0.38	46	2.10	60	8.99	5	1.72	—	—	139	13.19	115.5
Quercus rubra	—	—	1	0.03	2	0.48	4	1.56	3	2.59	10	4.66	19.7
Q. alba	—	—	—	—	2	0.26	4	2.31	1	0.85	7	3.42	16.7
Fraxinus americana	1	0.02	1	0.07	2	0.44	4	1.58	—	—	8	2.11	11.4
F. quadrangulata	1	0.02	3	0.12	4	0.77	2	0.71	—	—	10	1.62	10.6
Ulmus rubra	8	0.08	2	0.09	1	0.26	—	—	1	0.80	12	1.23	10.2
Celtis occidentalis	3	0.04	1	0.05	2	0.36	1	0.36	—	—	7	0.81	6.3
Q. macrocarpa	—	—	—	—	3	0.53	—	—	—	—	3	0.53	3.4
Carya ovata	—	—	—	—	1	0.22	1	0.44	—	—	2	0.66	3.3
Juglans nigra	—	—	—	—	1	0.22	—	—	—	—	1	0.22	1.3
Tilia americana	2	0.02	—	—	—	—	—	—	—	—	2	0.02	1.1
Carya tomentosa	1	0.01	—	—	—	—	—	—	—	—	1	0.01	0.5
Total	44	0.57	54	2.46	78	12.53	21	8.68	5	4.24	202	28.48	200.0

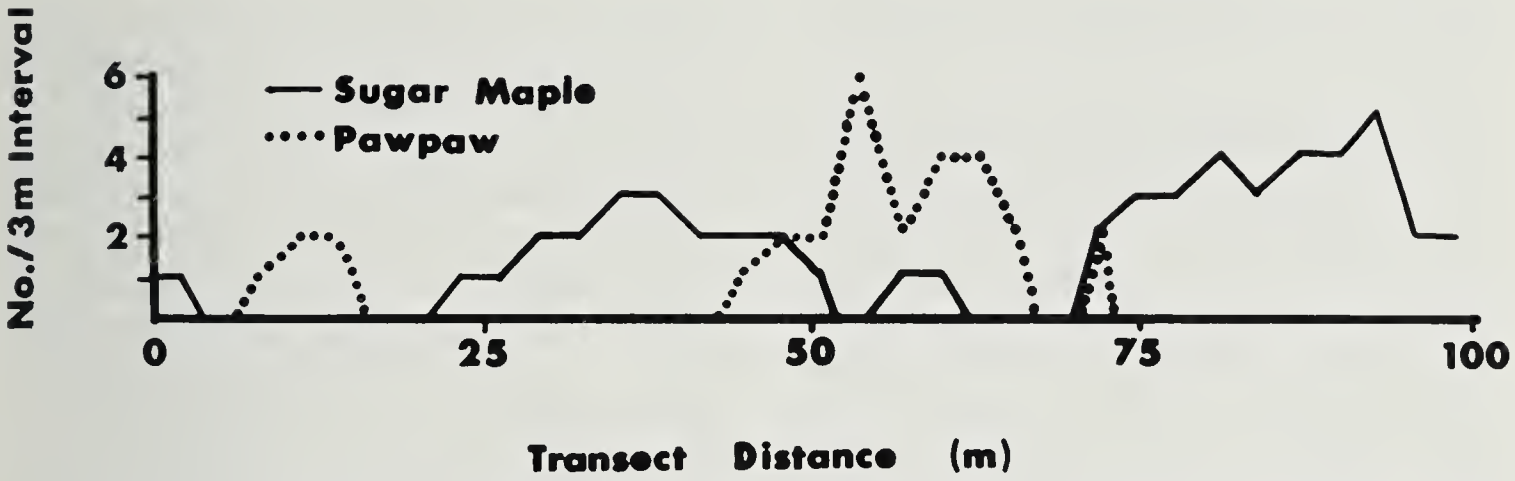

FIGURE 1.—Sapling distribution of sugar maple (*Acer saccharum*) and pawpaw (*Asimina triloba*) along a line intercept, showing their aggregated dispersion and negative association.

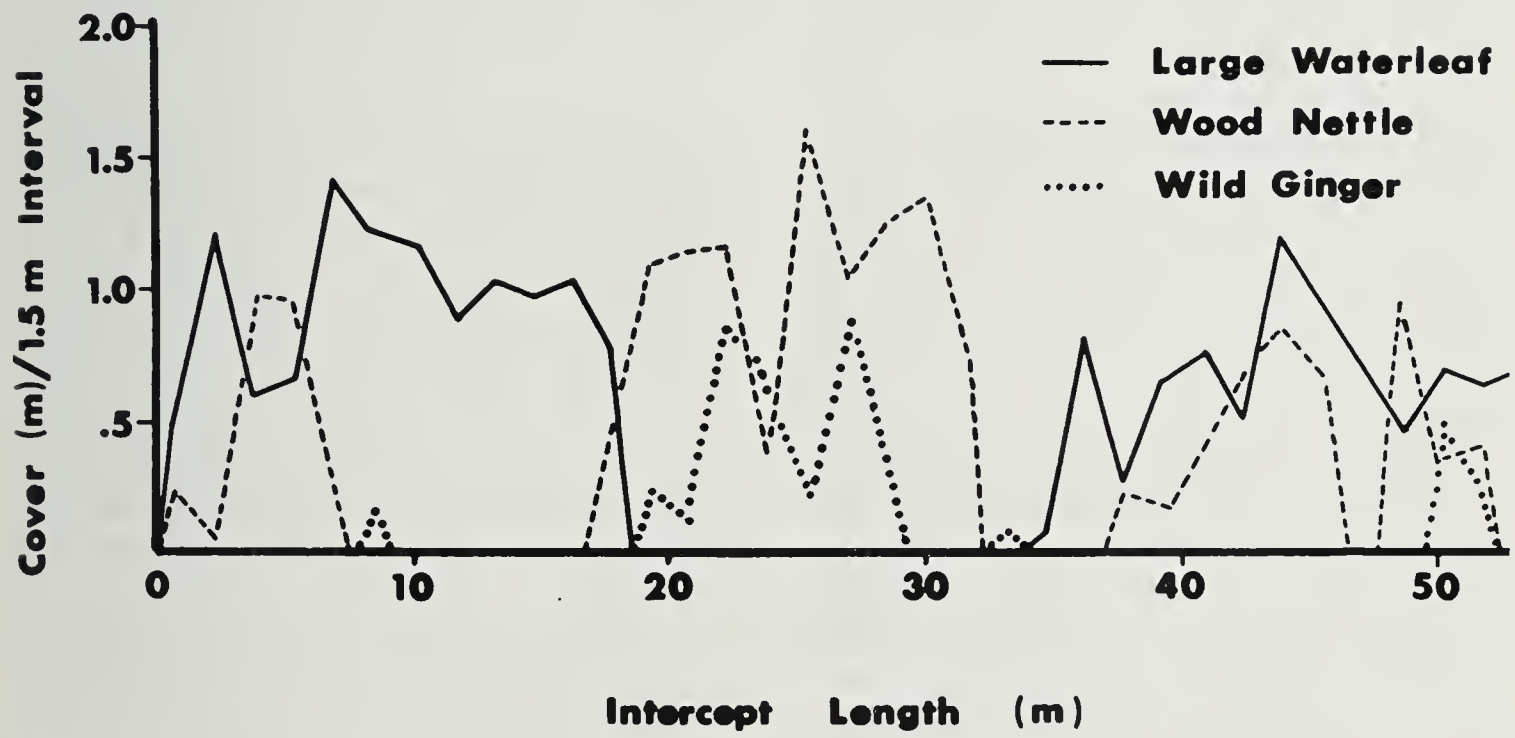

FIGURE 2.—Distribution of herb cover for the three dominant herbs, large waterleaf (*Hydrophyllum canadense*), wood nettle (*Laportea canadensis*), and wild ginger (*Asarum reflexum*), along a line intercept, showing their associations.

tive association and the latter a positive one, both significant at the .05 level.

The Simpson's diversity index calculated for our area was 2.04, and for the Funk Forest Natural Area (Boggess and Geis, 1966), 5.30. The coefficient of community between our area and Boggess and Geis' total area was .575, but between our area and their sugar maple community, .740.

DISCUSSION

Funk Forest is a mesic forest and *Acer saccharum* is the dominant species. Beech (*Fagus grandifolia*), characteristic of the Western Mesophytic Forest, is missing. Gleason (1912) in his study of several central Illinois prairie groves suggested that, in the past, burr oak (*Quercus macrocarpa*) was the leading dominant and that these forests had gone through three successional stages: oak-hickory to burr oak to red oak. He predicted the final stage of succession would be a maple-basswood type. Using Whittaker's (1953) definition of the term "climax", we concluded that the Funk Forest

area is now headed for a maple climax.

Few *Acer saccharum* seedlings were observed on the forest floor, and these were found where there were gaps in the canopy. In the matrix surrounding the gaps, *Quercus rubra* seedlings were more numerous than the *A. saccharum* seedlings. These findings are similar to those of Bray and Curtis' (1957) study of a maple-beech forest in Wisconsin. Our intrapopulation dispersion analysis of *A. saccharum* saplings indicated clumping, which further supports the observation that sugar maples arise in response to gaps in the canopy. Sugar maple does not establish seedlings extensively in the dense shade of a stable forest in which it is the dominant tree. The ability of *A. saccharum* to take advantage of canopy openings and to remain the dominant member of the forest community seems to depend on seed production and the ability of seedlings to grow rapidly in gaps and overtop those species already present (Bray, 1956.)

Sugar maples have replaced oaks as the dominant species. Table 1 shows that the majority of oak species are in the larger size classes, while sugar maple is present in all but the largest diameter class. As Gleason (1912) suggested, oaks were apparently the pioneer climax species, but as canopy space opens, they are being replaced by sugar maple. A diameter class comparison with Calef's (1953) and Boggess and Geis' (1966) data further shows the increase in sugar maple and decrease in oaks over the last two decades. Sugar maple has been increasing in all diameter classes, while oaks are decreasing in the larger diameter classes due to mortality and are not being replaced. Though *Quercus rubra* seedlings are prevalent in the stand, they persist only a year and usually do not develop into saplings (Table 2). The future status of the oaks suggests a decreasing importance as the forest approaches a sugar maple climax.

In 1953 Calef reported a maple-elm association in the area. Boggess and Geis (1966) noted a decrease in the importance of American elm (*Ulmus americana*) twelve years later. By 1970, we found no live American elm in our study area, though it was present in very limited numbers in the stand. Dead trees indicated its former presence. Dutch elm disease and phloem necrosis have caused the mortality of *U. americana*, and may also explain the decline of *U. rubra* in the stand.

The dispersion of sugar maple and oak trees was close to random, whereas the saplings were strongly clumped. Goodall (1952) pointed out that random distribution of individuals is less common than aggregation in natural vegetation. Whitford (1949) found that for

TABLE 2.—Sapling Analysis from Transect and Intercept Methods[a]

Species	Dens. (No./ha)	Rel. Dens.	Rel. Freq.	Rel. Dom.	Imp. Val.
Acer saccharum	3280	67.1	55.6	62.9	185.6
Asimina triloba	1100	22.5	22.2	11.0	55.7
Ulmus rubra	180	3.7	8.6	21.4	33.7
Carya ovata	210	4.3	8.6	3.3	16.2
Others[b]	120	2.4	5.0	1.4	8.8

[a]Density and frequency computed from transect intervals, and dominance from line intercept.

[b]*Cercis canadensis*, *Celtis occidentalis*, *Morus rubra*, *Carya tomentosa*, *Fraxinus quadrangulata*, and *Quercus rubra*.

many species young plants are aggregated but older ones approach random distribution. Curtis and McIntosh (1950) reported similar findings: mature trees tend to be randomly distributed, particularly in large size classes; saplings are more clumped; and seedlings show maximum aggregation.

Presently pawpaws (*Asimina triloba*) are the only saplings that approach maple saplings in importance (Table 2). The negative association between pawpaw and sugar maple saplings could be explained by interspecific competition. However, since *Asimina* is an understory tree which does well under minimum light and dense canopy, and reproduces primarily vegetatively; and *Acer* is found where there are canopy openings, and reproduces by seed, it is likely that this negative association reflects their different environmental requirements and their growth habits.

The dominant herbs were wild ginger (*Asarum reflexum*), wood nettle (*Laportea canadensis*), large waterleaf (*Hydrophyllum canadense*), and appendaged waterleaf (*H. appendiculatum*) (Table 3). Both Cain (1939) and Gleason (1926) pointed out the ambiguity of the term "association". Here we use the word to mean the coincident occurrence of the dominants. The wood nettle-wild ginger-appendaged waterleaf association was common throughout the area. McDougall (1922) reported this association to be typical of sugar maple forests: he used the term "symbiosis" to explain their interrelationships, but his definition of the term is no longer in current usage. Rowe (1956) found herbaceous undergrowth to be an accurate indicator of the tree climax in his area. Daubenmire (1968, p 221) suggested that forest undergrowth is commonly a rich mixture of species that persists indefinitely, so the present association may represent the dynamic equilibrium for the area.

Wild ginger is found throughout the stand, but due to its growth habit is not conspicuous. In spring the large waterleaf forms dense patches and does not regularly associate with the other herb dominants; in fact, it is negatively associated with wild ginger, appendaged waterleaf, and most of the less important herbs. In summer, wood nettle is found abundantly in moist areas of the woods except in areas of heavy canopy. A preliminary study has shown that light intensity is an important factor in the distribution of these herb dominants (manuscript in preparation).

The community similarity coefficients showed that our study area

TABLE 3.—Analysis of Herbs with an Importance Value over 5.0[a]

Species	Dens. (No./m²)	Rel. Dens.	Rel. Freq.	Rel. Dom.	Imp. Val.
Asarum reflexum	43.1	51.8	15.9	16.8	84.5
Laportea canadensis	14.7	17.6	15.9	45.9	79.4
Hydrophyllum canadense	8.6	10.3	2.1	28.4	40.8
H. appendiculatum	3.2	3.8	9.5	3.3	16.6
Smilacina racemosa	2.5	3.0	8.4	0.7	12.1
Osmorhiza longistylis	1.1	1.4	7.5	0.1	9.0
Quercus rubra (seedlings)	2.1	2.6	3.2	2.2	8.0
Cystopteris fragilis	1.6	1.9	5.3	0.5	7.7
Phryma leptostachya	1.9	2.3	5.3	—	7.6
Viola affinis	1.2	1.4	5.3	—	6.7
Others	3.0	3.9	21.6	2.1	27.6

[a]Density and frequency calculated by quadrant method, dominance by line intercept.

was most similar to the maple stand in the Funk Forest Natural Area (Boggess and Geis, 1966). The fact that the coefficient is not even higher reflects the continued loss of American elm and oaks during the intervening years. The low Simpson's diversity index supports our observation that the study area has a rather homogenous composition and was largely sugar maple. The Funk Forest Natural Area has a higher index value because it is a larger area and includes several different successional stages.

Acknowledgments

We wish to thank Dr. John D. Buffington for his continuing encouragement and guidance, and Dr. Birkenholz, Dr. Haines, and Dr. Liberta for their helpful suggestions in preparing the manuscript.

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Manuscript received August 27, 1971.

GLUCOSE INHIBITION OF PLASMODIAL MIGRATION IN THE ACELLULAR SLIME MOLD, *PHYSARUM POLYCEPHALUM*

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ABSTRACT.—Plasmodia of the acellular slime mold, *Physarum polycephalum*, were oriented in their migration over, first, a plain agar surface (2-1/2% in distilled water), secondly an agar surface containing a sugar or test compound, and lastly a plain agar surface. The distance migrated was recorded at timed intervals, and the morphology of the plasmodium was observed. It was found that glucose and mannose, but not fructose, interrupted migration, but did not change its direction. Various other carbon source compounds did not affect the migration pattern, but some, such as pyruvate, 3-phosphoglycerate, gluconate, and 6-phosphogluconate, while they affected the overall morphology, did not halt the migration. It is speculated that plasmodial movement is inhibited when various utilizable sugars are metabolized by a pathway involving a glucose oxidase. The resulting gluconate is further degraded by other unknown pathways, several possibilities of which are discussed.

No single theory is universally accepted for protoplasmic movement. The origin and control of the energy for movement are still unknown. Rhythmic protoplasmic streaming in the slime mold *Physarum polycephalum* is easily seen and has been much investigated with a view to correlating streaming with plasmodial movement (Kamiya, 1953). Camp (1936) described a consistent, easy method for culturing the mold, and Anderson (1963) devised a method for orienting starved, migrating plasmodia on an agar tract.

Investigations with slime mold plasmodia have implicated the Embden-Meyerhof pathway as powering streaming. Iodoacetic acid (IAA), which inhibits glyceraldehyde-3-phosphate dehydrogenase, reduced the motive force but did not impair respiration (Kamiya, 1959 and Ohta, 1954). Fluoride, an enolase and phosphoglucomutase inhibitor acted similarly. KCN and CO_2 decreased respiration but increased the motive force (Kamiya, 1959, Kamiya, et al, 1957 and Ohta, 1954). Ohta (unpubl.) found lactic acid dehydrogenase and glycogen in the mold. Therefore various workers (Hatano and Takeuchi, 1960; Kamiya, et al, 1957; and Ohta, 1954) concluded that the motive force derives from glycolysis.

Daniel (personal communication) was unable to demonstrate several Embden-Meyerhof enzymes in *Physarum*. Daniel & Rusch (1962), studying inhibition of sporulation of *Physarum* after exposure to light, found that glucose strongly inhibited sporulation, but fluoride and IAA did not. Pyruvate and lactate also inhibited sporulation but 3-phosphoglycerate did not. They concluded that pyruvate, if the effective metabolite of glucose, probably did not arise from glycolysis.

Miller (1965) noted that glucose as substrate made a migrating, oriented plasmodium stop. Daniel (1964) found that media supporting rapid growth suppressed migration, suggesting a competition between growth and motility.

This study describes how migrating plasmodia of *P. polycephalum* react to substrates, as evidenced by decrease or stoppage of migration, and the effect of utilized substrates on the appearance of the plasmodia.

If glycolysis is linked to the motive force then one would expect glucose, fructose, mannose, and certain glycolytic intermediates to inhibit migration. If alternative pathways operate, then perhaps glucose does not necessarily provide energy via glycolysis. Intermediates of alternate pathways, e.g., gluconic acid, 6-phosphogluconate or 2-ketogluconate should decrease migration rate or alter the morphological aspect of the plasmodium.

Materials and Methods

P. polycephalum was initially grown in shake culture (Daniel & Baldwin, 1964). After 3-days growth, the medium was decanted and the microplasmodia were then poured onto paper toweling supported on glass beads in a Petri dish. The plasmodia were washed in fresh culture medium, then kept at 21° C in an incubator until they fused. The plasmodia were then cultured by Camp's method for a period of three days (Camp, 1936).

As mentioned, migrating plasmodia slow and stop on encountering a utilizable substrate. Induction and study of oriented plasmodia have been described (Miller and Anderson, 1970).

When the Camp culture was completely covered with mold, the toweling was cut into small pieces and divided equally among the 3 tracks per tray. Each tray was covered with Saran Wrap to avoid dessication, then with black plastic sheeting to exclude light. Rates of migration were then observed. When a plasmodium had a well-organized front and was migrating at a uniform rate (approximately six hours after migration began), a slot of agar, volume 10 ml, was cut from the strip in front of the plasmodium. Small rectangular forms were fashioned from aluminum foil such that when filled to a depth of 0.5 cm they contained 10 ml; and into each was poured 8 ml of agar and 2 ml of a solution of the test substrate so that the final concentration of substrate and of agar in the 10 ml volume would be that desired. The small substrate-containing blocks were then placed into the slots in front of the migrating plasmodia and the effects observed as the organisms moved onto the substrates. The substrates employed are listed (Tables 1-4). One plasmodium of each set of 3 per tray was used as a control in which a small slot containing agar but no substrate was employed; in this way each plasmodium served as its own control.

Results

When 0.1, 0.05, or 0.005 M glucose was introduced as substrate, migrating plasmodia slowed, and although the advancing front passed

Table 1.—Effects of hexoses and hexonic acids on oriented migration of a plasmodium

Compounds	Conc. (moles)	Effect on migration	morphology	Effect on growth*
-D-Glucose	0.1, 0.05, 0.005	stop	sheet	+
-D-Glucose	0.05	stop	sheet	
Mannose	0.05, 0.005	stop	sheet	+
Galactose	0.05, 0.005	slowing	sheet	0
Gluconate	0.05, 0.005	none	lacy fan	0
6-P-Gluconate	0.005	none	lacy fan	
2-K-Gluconate	0.05	none	lacy fan	
Fructose	0.5, 0.05, 0.005	none	none	+

*from (Daniel and Baldwin, 1964).

TABLE 2.—Effects of inhibitors on oriented migration of a plasmodium

Compounds	Conc. (moles)	Effect on migration	morphology	Direction reversal
Glucosamine	0.05	none	none	+
2-Deoxyglucose	0.005	none	none	−
Citrate	0.05	stop	front disrupted	+
3-Aminotriazole	0.005	none	none	−
Caffeine	0.005	stop	none	+

TABLE 3.—Effects of inhibitors on oriented movement of a plasmodium when glucose is present

Compounds	Conc. (moles)	Effect on migration	morphology	Direction reversal
Glucosamine + Glucose	0.025 + 0.05	slowing	lacy fan	−
2-Deoxyglucose + Glucose	0.05 − 0.05	slowing	sheet	−
Citrate + Glucose	0.05 + 0.05	stop	front disrupted	+
3-Aminotriazole + Glucose	0.005 + 0.05	none	none	−
Caffeine + Glucose	2.5×10^{-3} to 2.5×10^{-5} + 0.05	±	variable	−

TABLE 4.—Effect of other substrates on oriented migration

Compound	Conc. (moles)	Effect on migration	morphology	Direction reversal
Sedoheptulose	0.05	none	none	−
Glyceraldehyde	0.05, 0.005	none	none	−
Pyruvate	0.05, 0.005	slowing	lacy fan	−
3-P-Glyceraldehyde	0.005	none	lacy fan	−
Pyruvate + 3-P-Glyceraldehyde	0.005 + 0.005	none	none	−

over the substrate slot and moved a few cm beyond, most of the mold piled up on the slot so that even after the front had reached the end of the track considerable mold remained on the slot. In all glucose tests the advancing front became a flattened sheet.

Plasmodia did not stop at 0.5, 0.05 or 0.005 M fructose. There was no change in the rate of migration and each advancing plasmodium seemed unchanged from the controls.

The response to mannose was qualitatively like that seen with glucose. A slowing of migration was not apparent as with glucose, but at both concentrations (0.05 and 0.005 M) there was considerable pile-up of mold on the substrate block long after the advancing front had moved on.

Sedoheptulose 0.05 M had no effect on migration rate or appearance of plasmodia.

Pyruvate profoundly affected the plasmodia. At 0.05 M the advancing

front stopped completely, then fanned out as a fine lacework over the substrate slot. Eventually the plasmodia regained their original appearance after leaving the slot and migrated to the end of the track. Pyruvate 0.005 M did not slow migration but did disrupt the front into a fan-like lacework, which disappeared after the plasmodia had traversed the substrate slot. In all cases the plasmodium had migrated nearly to the end of the track before fully regaining normal appearance.

The effect of 3-phosphoglyceric acid (3-PGA) 0.005 M was much like that of pyruvate 0.005 M. The migration rate was not perceptibly affected but there was a distinct shift to a lacework front which disappeared after passage of the plasmodium beyond the slot.

Glyceraldehyde 0.05 and 0.005 M had no detectable effect on migrating plasmodia.

Migrating mold reacted to gluconate at 0.05 and 0.005 M; with both, migration rate was unaltered but the appearance of the advancing front was. At each concentration, the front became a fan-like lacework not unlike that seen with pyruvate. The mold did not hesitate in its migration across the slot, and upon reaching the far side resumed a normal appearance. The effects of 6-phosphogluconate at 0.005 M on plasmodia paralleled those with gluconate. Migration rate was unaffected but the advancing front was altered in the same manner and resumed its normal appearance after traversing the substrate block.

Glucosamine 0.005 M did not affect migration rate or appearance of the plasmodium. When glucose (0.05 M) and glucosamine (0.025 M) were put into the same block, a dramatic change was noted: as the front moved onto the slot, it assumed a disrupted and lacy appearance, although the plasmodium continued to migrate. Upon reaching plain agar again the front resumed a normal appearance and continued moving to the end of the track, although some mold remained on the slot, as it had on the glucose experiments reported above.

Citrate 0.05 M profoundly changed the mold. On reaching the substrate block, the front stopped without moving onto the slot. The leading edge turned dark yellow and the mold remained immobile for several hours before reversing. When 0.05 M glucose was added to the citrate slot, the mold moved partly onto the block before stopping. Again the leading edge darkened but there was a pile-up of mold at the near edge of the block. Finally, the mold reversed direction, as seen with citrate alone.

Addition of 0.05 M 2-deoxyglucose (2-DG) to the agar slot affected neither migration rate nor morphology. If 0.05 M 2-DG and 0.05 M glucose were added, much of the mold stayed on the slot, as in experiments with glucose the sole substrate.

The response to 0.05 M galactose resembled that with glucose. The advancing front migrated to the end of the track but much of the mold piled up on the slot; however the effect was less pronounced than with glucose. This was demonstrated with 0.005 M galactose; very little mold remained on the slot.

3-Aminotriazole (3-AT) 0.005 M in the substrate slot did not affect migrating plasmodia. With glucose 0.05 M and 3-AT used together, very little mold remained on the slot: most of the mold migrated to the end of the track without reacting to the glucose.

2-Ketogluconate 0.05 M made the advancing front fan out into broad finger-like projections rather than the fine lacework seen with

gluconate. There was no hesitation in migration, however, and the front regained its integrity after traversing the substrate slot.

When equimolar pyruvate and 3-PGA (0.005 M) were used together in the substrate slot, the mold did not stop on the slot and there was no change in appearance of the advancing front.

Caffeine 0.005 M made the migrating plasmodium completely reverse although appearance was unaffected. When caffeine was mixed with 0.05 M glucose, the results depended upon the caffeine concentration. At high caffeine concentration (0.0025 M) migration direction was not reversed and the mold did not stop on the glucose slot, but migrated to the end of the track. At lower caffeine concentrations more mold piled up on the glucose slots until at caffeine 2.5×10^{-5} M the mold remained on the slot just as it did with glucose alone.

Discussion

The results reveal that at least 3 metabolic pathways are operating simultaneously and channeling energy to different processes, locomotion, growth and morphological change.

As shown, glucose makes a starved, oriented plasmodium stop; it also supports growth in shake culture (Daniel and Baldwin, 1964). Fructose, which according to these authors also supports growth very well in shake culture, does not affect migration rate or appearance (see

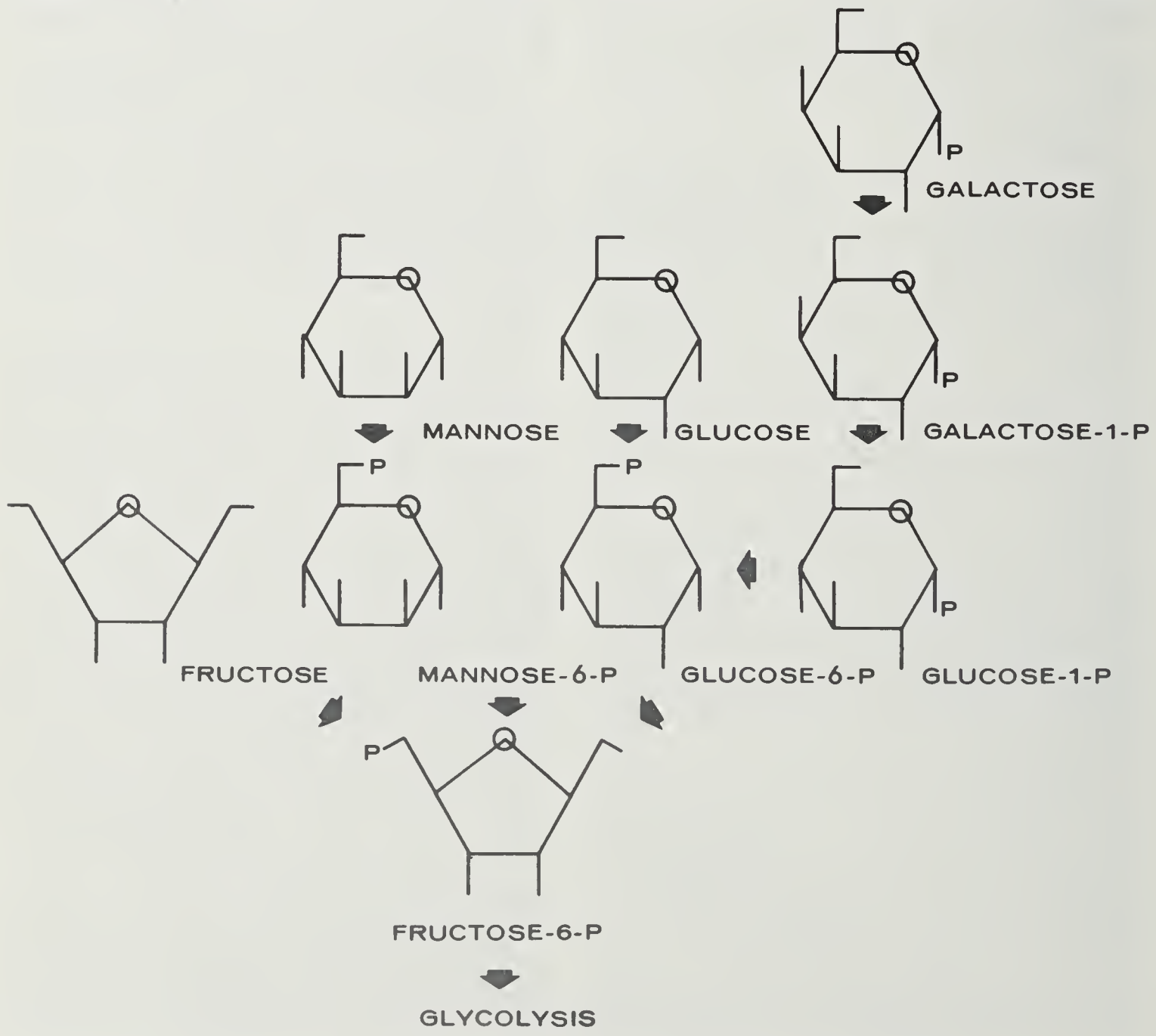

Figure 1.—Metabolic pathways to Fructose-6-phosphate.

Table 1). Mannose, which is first converted to fructose-6-phosphate (see Fig. 1) before glycolysis, supports growth at least as well as glucose in shake culture as demonstrated in our laboratory and is also nearly as effective as glucose in stopping a migrating plasmodium. Galactose, which Daniel and Baldwin (1964) found will not support growth in shake culture, will stop a migrating plasmodium, although not as effectively as does glucose. These results suggest glucose, mannose, and galactose enter a pathway related to movement but which will not accept fructose, and into another pathway, e.g., the Embden-Meyerhof, whose products are channeled to growth and which will accept fructose, glucose, and mannose.

If hexokinase activity is necessary for movement, one would expect the ability of the mold to react to glucose to diminish with increasing concentrations of glucosamine (Table 2), a competitive inhibitor of hexokinase, but even at equimolar glucose and glucosamine there was no evidence of impairment of glucose recognition by the mold (Table 3). A similar argument can be advanced for the lack of involvement in movement of the key glycolytic enzyme phosphofructokinase. Our results show that 2-deoxyglucose, which is easily phosphorylated by hexokinase, did not affect the reaction to glucose. Fig. 1 shows the routes of entry into glycolysis of the various sugars tested. Glucose, mannose, and fructose, all supporting growth, enter by very similar means, but galactose, which does not support growth, enters much more circuitously. If, then, glycolysis (at least that part which is beyond fructokinase), does not control the movement, other sugars may be metabolized via one or more of the various routes involving gluconic acid or its derivatives. Fig 2 depicts some possible metabolic paths postulated to exist in various bacteria and

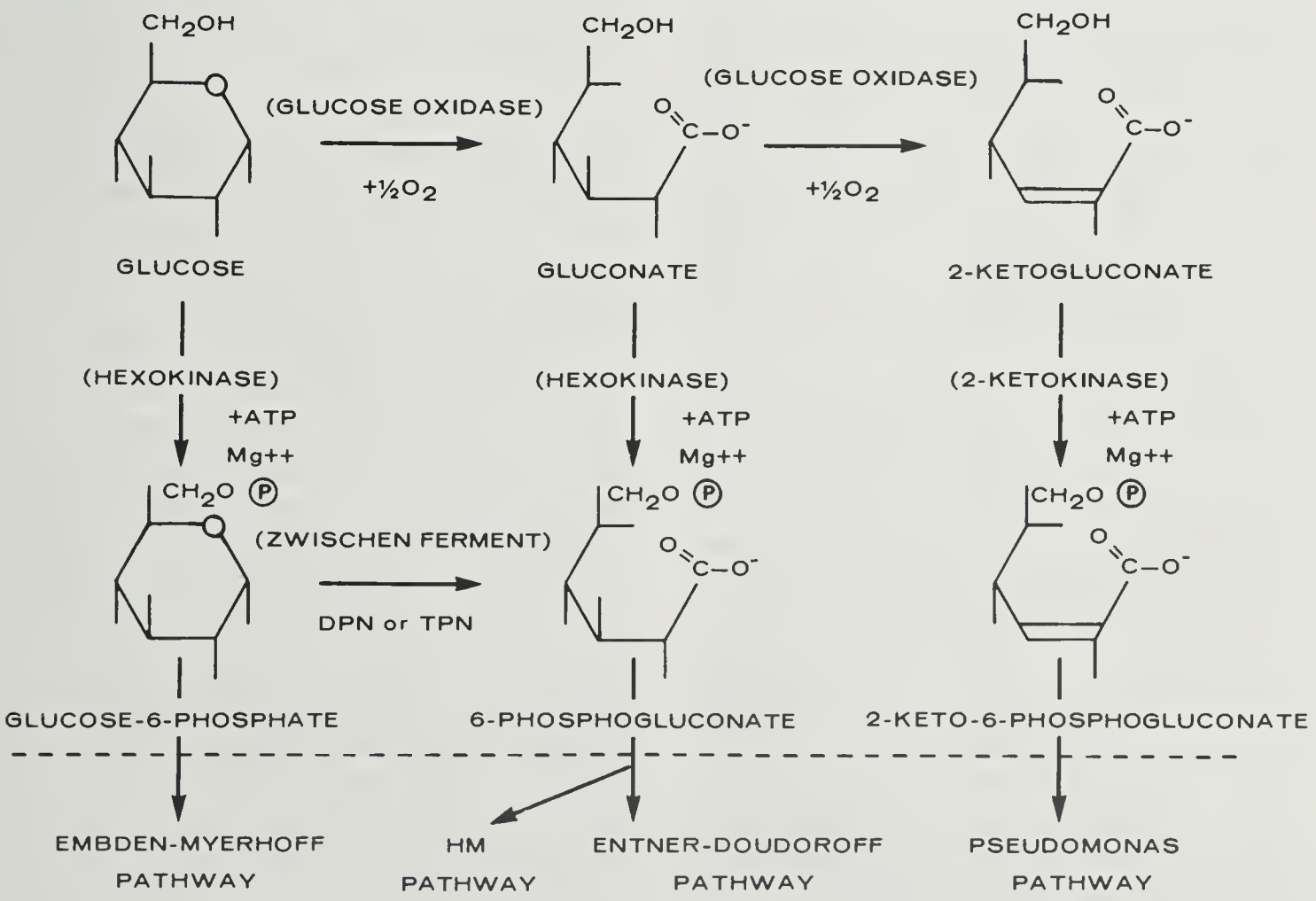

FIGURE 2.—Postulated metabolic paths.

fungi, as well as in other organisms. One possibility is for glucose-6-phosphate to be oxidized to 6-phosphogluconate by glucose-6-phosphate dehydrogenase and lactonase (Dickens, 1938). Two major pathways diverge from 6-phosphogluconate, the hexose monophosphate shunt and the Entner-Doudoroff pathway.

In the hexose monophosphate shunt 3-keto-6-phosphogluconate is produced from 6-phosphogluconate via 6-phosphogluconate dehydrogenase (Horecker and Smyrniotis, 1950), an enzyme inhibited by caffeine, Cu, and possibly IAA.

The mold was sensitive to gluconate and 6-phosphogluconate, although only morphology seemed affected; sedoheptulose and glyceraldehyde were inert. This raises a question as to whether this pathway is important for movement; this evidence is far from conclusive since neither compound was tested in phosphorylated form. Perhaps the mold cannot carry out the necessary phosphorylation steps, even if it used intermediates in the pathway. Use of phosphorylated test compounds might not resolve the issue since most phosphorylated sugars pass through membranes with great difficulty, if at all. The use of various inhibitors of the pathway provides some clarification. The fact, noted earlier, that glucosamine did not alter stoppage of the mold on glucose casts doubt on the importance of the initial part of the pathway since less glucose-6-phosphate would be formed. Also, 2-deoxyglucose, which competes reversibly with glucose for sites on glucose-6-phosphate dehydrogenase, had no ability, even at 0.05 M, to impair recognition of glucose.

In the Entner-Doudoroff pathway 6-phosphogluconate is converted by glutathione and Fe^{++} to 2-keto-3-deoxy-6-phosphogluconate (KDPG), which is then split into pyruvate and 3-phosphoglyceraldehyde (3-PGA1) by KDPG aldolase. The mold reacted to pyruvate, which slows down migration and alters morphology, and recognizes 3-phosphoglycerate (3-PGA), which alters morphology and is directly derivable from 3-PGA1.

Since the Dickens reaction apparently is not present to give rise to 6-phosphogluconate, there is most likely an alternative. Glucose can be oxidized to gluconic acid by at least 2 paths: a glucose oxidase system, or a cytochrome system (Wood and Schwerdt, 1953).

The cytochrome system mediates 2 successive oxidations on the substrate and yields 2-ketogluconate, and eventually yields 2 moles of pyruvate per mole of substrate. Results on 2-ketogluconate as a substrate for the mold were inconclusive. No effect on migration rate was observed, and the morphological pattern was not the usual lace pattern, but rather broad fingers. Quite possibly a pH effect was involved rather than actual substrate uptake. This system is dependent upon cytochromes which are very sensitive to cyanide (Wood and Schwerdt, 1953) and Kamiya (Kamiya, et al, 1957) showed that cyanide promoted motility.

The other possibility—that glucose oxidase operated—seems the likelier alternative. Several pyranose sugars, including glucose, galactose, mannose, and various disaccharides, are accepted by glucose oxidases, but furanoses e.g., fructose, apparently are not. The oxidase acting on its substrate yields gluconate and hydrogen peroxide which must be removed by catalase for the reaction to continue. Catalase is inhibited by 3-aminotriazole (and glucose utilization is impaired) and, as the results show, a mixture of glucose + 3-AT can-

not stop an advancing plasmodium, suggesting a breakdown of the link between the control mechanism of migration and substrate uptake. Gluconic acid produced by this system is phosphorylated by gluconokinase to 6-phosphogluconate. It is thus possible to generate this compound from glucose and for both the Entner-Duodoroff pathway and the hexose monophosphate shunt to operate even in the presence of a large concentration of either of the inhibitors 2-deoxyglucose or glucosamine. The fact that fluoride and IAA inhibit motility is not inconsistent. Fluoride inhibits KDPG aldolase as well as enolase (Entner and Duodoroff, 1952). If the motive force is somehow linked to this path it would be decreased by fluoride. The enzyme 6-phosphogluconate dehydrogenase is inhibited by a number of substances, among which are caffeine, Cu, and IAA (Scott and Cohen, 1953). Provocatively, Cu will make a migrating plasmodium reverse direction and become vacuolated (Park and Robinson, 1967).

The effects of caffeine are more complicated. The effects seen with caffeine + glucose suggest competitive inhibition: with increasing caffeine, glucose was less effective in stopping the mold. Caffeine alone, however, induced an extremely rapid reversal of direction of migration and actually speeded movement. This is difficult to reconcile with a simple blockage of 6-phosphogluconate dehydrogenase. The methyl xanthines are intimately related to the levels of cyclic 3′, 5′-adenosine monophosphate (cAMP) found in mammalian cells. Caffeine increases glycogenolysis, increasing availability of glucose from glycogen. Since cAMP is a high-energy compound whose free energy of hydrolysis exceeds that of ATP (Greengard, et al, 1969), it is possible that it is in some manner linked to the motive force, and caffeine in the substrate slot could increase cAMP levels and thus increase the motive force. That amoebae of *Dictostelium discoideum* are attracted to cAMP has already been shown (Konijn, et al, 1967), and Barkley (1969) found that the same organism secretes it. cAMP, in very low concentrations, increased movement of the amoebae of *Polysphondylium pallidum* (Konijn, et al, 1968).

The experiments on equimolar concentrations of pyruvate and 3-PGA invite speculation. Although each substrate alone affected the appearance of the mold, together they did not change the pattern of migration or morphology. Presumably the morphology of the organism reflects the relative rates of several pathways: then any compound altering relative rates might change morphology. If pyruvate and 3-PGA are involved, either would alter the relative rates. But were a system which normally produces equal amounts of the substances in question present, then addition of equimolar amounts of the two would not affect the relative rates of subsequent metabolic systems and morphology would be unaffected. Our evidence suggests that the link between glucose uptake and decrease of migration rate must be sited before the split of 2-keto-3-deoxy-6-phosphogluconate. The link may be associated with glucose oxidase because gluconate affects morphology but does not stop migration whereas glucose stops migration but does not affect morphology.

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Manuscript received July 12, 1971.

THE HYDROLYSIS OF ADENOSINE TRIPHOSPHATE BY BLUEGILL LIVER MITOCHONDRIA IN THE PRESENCE OF 2,4,5-T AND SILVEX DERIVATIVES

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ABSTRACT.—The effect of several phenoxy herbicides on the hydrolysis of adenosine-triphosphate (ATP) by bluegill, *Lepomis macrochirus*, in the presence of cadmium, zinc, manganese, magnesium and calcium were investigated. All the 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-(2,4,5-trichlorophenoxy) propionic acid (silvex), except the sodium salt, inhibited the hydrolysis of ATP in the presence of cadmium and zinc. The butyl ester of 2,4,5-T and the butoxy-ethanol and propyleneglycolbutylether esters of silvex increased the hydrolysis of ATP in the presence of manganese. The butyl ester of 2,4,5-T and the butoxy-ethanol ester of silvex increased the hydrolysis of ATP in the presence of calcium. The data suggest that various phenoxy derivatives can alter the hydrolysis of ATP and that the butoxy-ethanol, the butyl, and the isooctyl esters of 2,4,5-T and the butoxy-ethanol and propyleneglycolbutylether esters of silvex could alter oxidative phosphorylation in the presence of magnesium by their effects on the hydrolysis of ATP or other high energy phosphate intermediates.

After we had initiated our estimation of the toxicities of various aquatic herbicide formulations to small bluegills, *Lepomis macrochirus*, it was observed that different formulations containing various 2,4 dichlorophenoxyacetic acid derivatives, 2,4-D, had different levels of toxicity to small bluegills (Hiltibran 1967: Hughes and Davis 1963). We initiated biochemical studies to investigate the effects of possible pollutants on the succinic oxidase, alpha-keto-glutarate oxidases, and the hydrolysis of adenosinetriphosphate (ATP) by bluegill liver mitochondria. The effects of nine derivatives of 2,4-D on these enzyme complexes have been reported (Hiltibran 1969a, 1969b), and it was found that the 2,4-D derivatives which were the most toxic to bluegills altered either oxygen or phosphate uptake.

We continued the investigation of the effects of phenoxy derivatives by estimating the effects of the sodium salt, the butoxy-ethanol (BE), butyl, and isooctyl esters of 2,4,5-trichlorophenoxyacetic acid, and the sodium salt, the BE and propyleneglycolbutylether (PGBE) esters of 2-(2,4,5-trichlorophenoxy) propionic acid, silvex, on the succinic and alpha-ketoglutarate oxidases of bluegill liver mitochondria. It was found that these derivatives altered either oxygen or phosphate metabolism or both (Hiltibran 1972a).

Since the BE ester of 2,4,5-T, in the presence of succinate, and the BE ester of silvex, in the presence of alpha-ketoglutarate, did not alter oxygen metabolism but severely altered phosphate uptake, we investigated the effects of the 2,4,5-T and silvex derivatives on the hydrolysis of ATP.

METHODS

Native wild bluegills were held for two weeks at 25° C in aerated laboratory aquaria prior to use, and all enzyme assays were conducted at the same temperature. The procedures for the preparation of the mitochondria, for estimating the rate of release of inorganic phosphate from ATP, and for estimating the nitrogen content of the mitochondrial preparations have been reported (Hiltibran and Johnson 1965). The quantities of inorganic

phosphate released from the ATP were converted to micromoles of ATP hydrolyzed per milligram of nitrogen (micromoles ATP/hr/mg N). The data are the average values from three or more experiments and have been corrected for endogenous activity and the effects of solvents. The phenoxy derivatives were obtained from various manufacturers and were used without further purification. Ethyl alcohol or acetone redistilled in all-glass apparatus was used as a solvent.

Results

The effects of the sodium salt, butyl ester, BE ester, isooctyl ester and acid 2,4,5-T, and the sodium salt, the BE, and PGBE esters of silvex on the hydrolysis of ATP in the presence of cadmium are summarized in Table 1. The sodium salt 2,4,5-T did not alter the hydrolysis in the presence of cadmium. The acid and the butyl, BE, and isooctyl esters of 2,4,5-T and the sodium salt and the BE and the PGBE esters of silvex inhibited the hydrolysis of ATP.

The effects of the 2,4,5-T and silvex derivatives used in this investigation on the hydrolysis of ATP in the presence of zinc are shown in Table 2. The sodium salt of 2,4,5-T and the BE ester of silvex did not appreciably alter the hydrolysis of ATP. All of the other

Table 1.—The Effects of Phenoxy Derivatives on the Hydrolysis of ATP in the Presence of Cadmium

2,4,5-T Derivative	umoles/ml of Reaction Medium 1.5	2.5	5.0
	Average Change in umoles ATP/hr/mg N		
Free acid	±39	−22	−47
#exp.	(4)	(4)	(4)
Range	((+)6 −(−)124)	((−)4 −(−)44)	((−)4 −(−)(116)
Na salt	±24	±17	±37
#exp.	(4)	(3)	(4)
Range	((−)30 −(+)37)	((+)19 −(−)30)	((+)8 −(−)59)
Butoxy-ethanol ester	−43	−61	−67
#exp.	(3)	(3)	(3)
Range	((−)21 −(−)77)	((−)21 −(−)101)	((−)23 −(−)117)
Butyl ester	−29	−33	−16
#exp.	(5)	(4)	(5)
Range	((−)13 −(−)97)	((−)17 −(−)92)	((−)2 −(−)49)
Isooctyl ester	−16	−33	−10
#exp.	(5)	(5)	(4)
Range	((−)2 −(−)35)	((−)6 −(−)93)	((−)9 −(−)19)
Silvex Derivative			
Free acid	—	—	—
#exp.			
Range			
Na salt	−21	−37	−47
#exp.	(5)	(5)	(5)
Range	((−)13 −(−)30)	((−)20 −(−)52)	((−)31 −(−)54)
Butoxy-ethanol ester	−12	−28	−59
#exp.	(4)	(4)	(4)
Range	((−)2 −(−)23)	((−)10 −(−)35)	((−)24 −(−)157)
PGBE	−13	−55	−38
#exp.	(4)	(4)	(4)
Range	((−)3 −(−)23)	((−)15 −(−)105)	((−)14 −(−)59)

TABLE 2.—The Effects of Phenoxy Derivatives on the Hydrolysis of ATP in the Presence of Zinc

2,4,5-T Derivative	umoles/ml of Reaction Medium 1.5	2.5	5.0
	Average Change in umoles ATP/hr/mg N		
Free acid	−28	−31	−35
#exp.	(3)	(3)	(3)
Range	((−)12−(−)41)	((−)24−(−)39)	((−)28−(−)41)
Na salt	±8	±19	±12
#exp.	(5)	(5)	(5)
Range	((−)20−(+)5)	((−)27−(+)30)	((−)29−(+)3)
Butoxy-ethanol ester	±8	−27	−37
#exp.	(3)	(3)	(3)
Range	((+)15−(−)30)	((−)7−(−)45)	((−)12−(−)53)
Butyl ester	−16	− 6	−12
#exp.	(5)	(5)	(5)
Range	((−)5−(−)24)	((−)1−(−)16)	((−)6−(−)14)
Isooctyl ester	−18	−13	−14
#exp.	(5)	(6)	(6)
Range	((−)4−(−)22)	((−)5−(−)26)	((−)2−(−)28)
Silvex Derivative			
Free acid	—	—	—
#exp.			
Range			
Na salt	−16	−6	−24
#exp.	(4)	(4)	(5)
Range	((−)1−(−)36)	((−)2−(−)13)	((−)8−(−)59)
Butoxy-ethanol ester	±14	±14	−10
#exp.	(4)	(4)	(4)
Range	((+)3−(−)38)	((−)15−(+)25)	((−)5−(−)22)
PGBE	−27	−25	−33
#exp.	(3)	(3)	(3)
Range	((−)11−(−)82)	((−)13−(−)34)	((−)16−(−)71)

derivatives inhibited the hydrolysis of ATP to some extent, which ranged from 20 percent for the butyl and isooctyl esters of 2,4,5-T to approximately 50 percent at high concentrations (0.10) of the PGBE ester and the sodium salt of silvex and the free acid of 2,4,5-T.

The effects of 2,4,5-T and silvex derivatives on the hydrolysis of ATP in the presence of manganese are shown in Table 3. The sodium salt of 2,4,5-T did not alter the hydrolysis of ATP. The butyl and isooctyl esters of 2,4,5-T and the BE and PGBE esters of silvex increased the hydrolysis of ATP.

The effects of these derivatives of 2,4,5-T and silvex on the hydrolysis of ATP in the presence of magnesium are summarized in Table 4. The sodium salts of 2,4,5-T and silvex did not appreciably affect the hydrolysis of ATP. All of the other derivatives used increased the hydrolysis of ATP. The PGBE ester increased the hydrolysis of ATP approximately 100 percent, as did the butoxy-ethanol ester of 2,4,5-T. The isooctyl ester of 2,4,5-T and the butoxy-ethanol ester of silvex increased the hydrolysis of ATP more than did any other derivatives.

The effects of 2,4,5-T derivatives on the hydrolysis of ATP in the presence of calcium are summarized in Table 5. The sodium salt and BE and acid derivatives of 2,4,5-T

TABLE 3.—The Effects of Phenoxy Derivatives on the Hydrolysis of ATP in the Presence of Manganese

2,4,5-T Derivatives	umoles/ml of Reaction Medium 1.5	2.5	5.0
	Average Change in umoles ATP/hr/mg N		
Free acid	±21	±22	±26
#exp.	(4)	(4)	(4)
Range	((−)9 −(+)57)	((−)1 −(+)54)	((−)8 −(+)59)
Na salt	−13	−28	−28
#exp.	(5)	(4)	(5)
Range	((−)6 −(−)21)	((−)10 −(−)53)	((−)19 −(−)35)
Butoxy-ethanol ester	±33	±5	−9
#exp.	(3)	(3)	(3)
Range	((−)6 −(+)66)	((−)6 −(+)7)	((−)6 −(−)14)
Butyl ester	+21	+40	+42
#exp.	(5)	(7)	(6)
Range	((+)11 −(+)47)	((+)3 −(+)91)	((+)3 −(+)101)
Isooctyl ester	±17	+59	+32
#exp.	(7)	(5)	(6)
Range	((−)19 −(+)42)	((+)11 −(+)133)	((+)6 −(+)64)
Silvex Derivative			
Free acid	±27	−34	−24
#exp.	(4)	(4)	(5)
Range	((−)42 −(+)43)	((−)19 −(−)74)	((−)21 −(−)44)
Na salt	−11	−21	−34
#exp.	(4)	(4)	(5)
Range	((−)3 −(−)24)	((−)12 −(−)31)	((−)9 −(−)51)
Butoxy-ethanol ester	+59	+63	+109
#exp.	(4)	(4)	(4)
Range	((+)10 −(+)88)	((+)28 −(+)129)	((+)75 −(+)162)
PGBE	+54	+41	+9
#exp.	(3)	(3)	(3)
Range	((+)45 −(+)69)	((+)21 −(+)70)	((+)2 −(+)25)

and the sodium salt and the PGBE ester and acid derivative of silvex did not appreciably alter the hydrolysis of ATP. The butyl ester of 2,4,5-T and the BE ester of silvex increased the hydrolysis of ATP approximately 90 and 100 percent, respectively.

DISCUSSION

It is apparent from these results that the same derivatives of 2,4,5-T and silvex altered the hydrolysis of ATP in the presence of the various metals.

In a previous study it was found that the number and position of the chloro groups substituted on the phenoxyacetic or phenoxypropionic acid, when assayed as the sodium salts, did not alter the hydrolysis of ATP (Hiltibran 1972*b*). In a related study it became apparent that the effects of phenoxy derivatives on oxidative phosphorylation by bluegill liver mitochondria appeared to be the result of at least three factors: 1) the number and position of the chloro groups, 2) the salt or ester derivative, and 3) the acetic or propionic acid analog used (Hiltibran 1972*a*) (1972*b*). The effects of a specific phenoxy derivative on oxidative phosphorylation by bluegill liver mitochondria appeared to be the result of the interaction of each phenoxy derivative and the specific oxidative bluegill liver en-

TABLE 4.—The Effects of Phenoxy Derivatives on the Hydrilysos of ATP in the Presence of Magnesium

2,4,5-T Derivative	umoles/ml of Reaction Medium 1.5	2.5	5.0
	Average Change in umoles ATP/hr/mg N		
Free acid	±18	+43	+41
#exp.	(4)	(4)	(4)
Range	((−)10−(+)31)	((+)6−(+)79)	((+)8−(+)68)
Na salt	± 3	± 3	± 6
#exp.	(4)	(3)	(4)
Range	((−)2−(+)6)	(0−(+)6)	((−)7−(+)12)
Butoxy-ethanol ester	+ 9	+36	+33
#exp.	(3)	(3)	(3)
Range	((+)2−(+)13)	((+)7−(+)65)	((+)6−(+)55)
Butyl ester	±18	+25	+56
#exp.	(6)	(5)	(4)
Range	((+)10−(−)57)	((+)3−(+)45)	((+)10−(+)112)
Isooctyl ester	±14	+33	+34
#exp.	(6)	(6)	(6)
Range	((+)2−(−)56)	((+)3−(+)97)	((+)1−(+)65)
Silvex Derivative			
Free acid	± 6	±19	±13
#exp.	(4)	(4)	(5)
Range	((+)8−(−)10)	((+)2−(−)41)	((+)7−(−)28)
Na salt	± 9	± 5	±12
#exp.	(4)	(4)	(4)
Range	((−)6−(+)27)	((+)5−(−)8)	((−)11−(+)18)
Butoxy-ethanol ester	+12	+42	+107
#exp.	(4)	(4)	(4)
Range	((+)6−(+)26)	((+)16−(+)81)	((+)55−(+)165)
PGBE	±10	+26	+19
#exp.	(4)	(3)	(4)
Range	((−)1−(+)19)	((+)8−(+)60)	((+)4−(+)36)

zyme complex. When any one of the three parts was changed, the effect observed was also different. It was suggested that the chloro groups substituted on the phenoxy compounds conferred on those compounds their ability to alter the oxygen uptake by bluegill liver mitochondria. It was further suggested that this basic effect could be modified by the salt or ester derivative substituted on the carboxyl group and by either the acetic or propionic acid analog (Hiltibran 1972*a*). It would appear that a very similar situation occurred with the hydrolysis of ATP by bluegill liver mitochondria.

The basic effect of either 2,4,5-T or silvex on the hydrolysis of ATP will be considered the effects of the acid of 2,4,5-T or silvex derivatives and hereafter will be referred to as either 2,4,5-T or silvex. In the presence of magnesium, the hydrolysis of ATP was increased in the presence of 2,4,5-T, but the sodium salt did not alter the hydrolysis of ATP. The presence of a BE, butyl, or isooctyl analog on the 2,4,5-T did not further alter the basic effect of 2,4,5-T. Silvex or the sodium salt of silvex (Table 4) did not alter the hydrolysis of ATP; however, the presence of the BE or PGBE analog on the silvex increased the hydrolysis of ATP. The BE ester was more

TABLE 5.—The Effects of Phenoxy Derivatives on the Hydrolysis of ATP in the Presence of Calcium

2,4,5-T Derivative	umoles/ml of Reaction Medium 1.5	2.5	5.0
	Average Change in umoles ATP/hr/mg N		
Free acid	±11	±17	±17
#exp.	(4)	(4)	(4)
Range	((+)10 −(−)27)	((−)26 −(+)32)	((+)24 −(−)36)
Na salt	±4	±3	−4
#exp.	(4)	(4)	(3)
Range	((−)4 −(+)6)	((+)2 −(−)5)	((−)2 −(−)6)
Butoxy-ethanol ester	±4	±16	±7
#exp.	(3)	(3)	(3)
Range	((−)4 −(+)5)	((+)2 −(+)36)	((−)3 −(+)18)
Butyl ester	±4	+9	+22
#exp.	(6)	(6)	(3)
Range	((+)2 −(−)9)	((+)1 −(+)14)	((+)5 −(+)35)
Isooctyl ester	−3	+14	±9
#exp.	(6)	(6)	(6)
Range	(−)2 −(−)9)	((+)2 −(+)42)	((−)5 −(+)17)
Silvex Derivative			
Free acid	±3	±10	±7
#exp.	(4)	(4)	(5)
Range	((+)4 −(−)5)	((+)1 −(−)37)	((+)4 −(−)12)
Na salt	±7	±7	±6
#exp.	(4)	(4)	(4)
Range	((−)4 −(+)15)	((−)4 −(+)14)	((−)8 −(+)8)
Butoxy-ethanol ester	+12	+55	+32
#exp.	(4)	(4)	(4)
Range	((+)2 −(+)25)	((+)2 −(+)140)	((+)14 −(+)69)
PGBE	±5	±14	±7
#exp.	(4)	(3)	(4)
Range	((−) −(+)11)	((−) −(+)21)	((−)1 −(+)14)

effective than the PGBE ester of silvex.

A discussion of all the data will not be undertaken, but two additional points will be noted. In the presence of manganese (Table 3) 2,4,5-T and the BE ester did not alter the hydrolysis of ATP. The sodium salt inhibited the hydrolysis of ATP, but the butyl and isooctyl ester increased the hydrolysis. When the propionic acid analog was used, different effects were noted. In the presence of calcium, 2,4,5-T, sodium salt and BE ester did not alter the hydrolysis of ATP, but the hydrolysis of ATP was increased by the butyl and isooctyl esters. In the presence of the propionic acid analog, only the BE ester altered the hydrolysis of ATP (Table 5).

Additional comparisons are possible when the effects of the similar esters of 2,4-D, 2,4,5-T, and silvex are made. For example, in the presence of magnesium, the BE ester of 2,4-D did not alter the hydrolysis of ATP (Hiltibran 1969*a*), but the BE esters of 2,4,5-T and silvex increased the hydrolysis of ATP. The greater effect on the hydrolysis of ATP occurred with the BE ester of silvex.

These data extend the observations of the effects of various phenoxy derivatives to five additional enzyme activities of the bluegill

liver mitochondria and also suggest that the interaction of any phenoxy derivative with any bluegill liver enzyme complex is an interaction of each specific phenoxy derivative with each enzyme complex. Previously it was suggested that the various phenoxy derivatives altered oxidative phosphorylation by bluegill liver mitochondria by their effect on oxygen uptake (Hiltibran 1969*b* and 1972*a*). These data indicate that many of these phenoxy derivatives could affect oxidative phosphorylation of bluegill liver mitochondria by altering the hydrolysis of ATP. The BE ester of 2,4,5-T did not alter the uptake of oxygen in the presence of succinate as substrate, but phosphate uptake was altered (Hiltibran 1971*a*). The BE ester of silvex did not alter oxygen uptake in the presence of alpha-ketoglutarate as substrate, but phosphate uptake was altered. The BE ester of 2,4,5-T and silvex increased the hydrolysis of ATP in the presence of magnesium, suggesting that that these phenoxy derivatives could alter oxidative phosphorylation by bluegill liver mitochondria by their effects on phosphate metabolism. However, it appears that the primary effects of most of the phenoxy derivatives on the bluegill liver mitochondria oxidative phosphorylation are on the oxygen metabolism (Hiltibran 1972*a*). These data also support the point of view that the various phenoxy compounds can cause cellular biochemical alterations in bluegill tissue and these alterations might be related to the toxic action of these compounds to bluegills.

Acknowledgments

The author acknowledges the technical assistance of Charlene Mathis, Karen Mettling, Stephen Forbes, William Maierhofer, Douglas Schemske, Richard Schumacher, and Robert Techlaw.

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Manuscript received July 19, 1971.

INVASION OF WOODY PLANTS IN ABANDONED SOIL BANK AREA OF PINE ROCK PRESERVE IN OGLE COUNTY

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ABSTRACT.—Three plots totaling 21 acres of previously cultivated land in Pine Rock Preserve east of Oregon in Ogle County were designated as "soil bank" in 1963, planted to domestic grasses and legumes, and mowed for the last time in 1964. In the six growing seasons since 1964 there has been extensive invasion by prairie grasses and forbs from the adjacent well-developed prairie. There has been scattered invasion by woody plants. Of twenty-two species of woody plants identified in the study area, fourteen produce fruits commonly eaten by birds. This group includes two species of trees, two of vines, and ten of shrubs. Of eight additional trees, seven are disseminated by wind and one (black oak) by squirrels. Power lines supported by crossbars on double poles traverse one of the plots and a nearby prairie. These resting places for birds have resulted in a high concentration of woody plants around the poles and a greater density of bird-introduced woody plants under the lines than in the open fields. Thus the presence of a factor introduced by man seems to have a definite effect in speeding up the invasion of prairie and abandoned field by woody plants, especially shrubs.

Land which is lying dormant after a period of agricultural use characteristically undergoes secondary successional changes (Oosting, 1956). Man often interferes with nature's method of restoring land, and since that influence is not always beneficial, it becomes both interesting and necessary to consider what that influence is and what effect it has.

An area where this is particularly evident is Pine Rock Preserve near Oregon, Illinois. A portion of this preserve was agricultural land which has been out of production since 1964. Man's influence is evident in the electrical high lines which bisect the preserve diagonally, in a secondary electrical line located along the west side of the preserve and in fences in and around the preserve. Although the current herbaceous stage of succession consists primarily of prairie species typical for this area, woody species have invaded into the prairie and old field areas. Bullington (1970) has indicated that pioneer trees in open pasture are usually those with seeds transported by wind or birds.

The purpose of this study is to determine to what extent the poles and lines have had an influence on the introduction of woody species into abandoned farm land as compared to random invasion in similar areas not influenced by these resting places for birds. In addition, since there is a possibility of developing a portion of the preserve as a prairie, this report will indicate the status of the area at this time.

DESCRIPTION OF THE PRESERVE

Pine Rock Preserve is situated east of Oregon, Illinois, on Illinois Route 64. It is located on the Oregon quadrangle map in the northeastern and northwestern quarters of Section 8 of Pine Rock Township (T. 23 N., R. 11 E.), Ogle County, Illinois.

A brief history of the area follows. The double poles and power line bisecting the area south of highway 64 and running from northeast to southwest (see Figure 1) were installed in the early 1920's (at least by 1925). After being pastured in the early 1930's, the whole

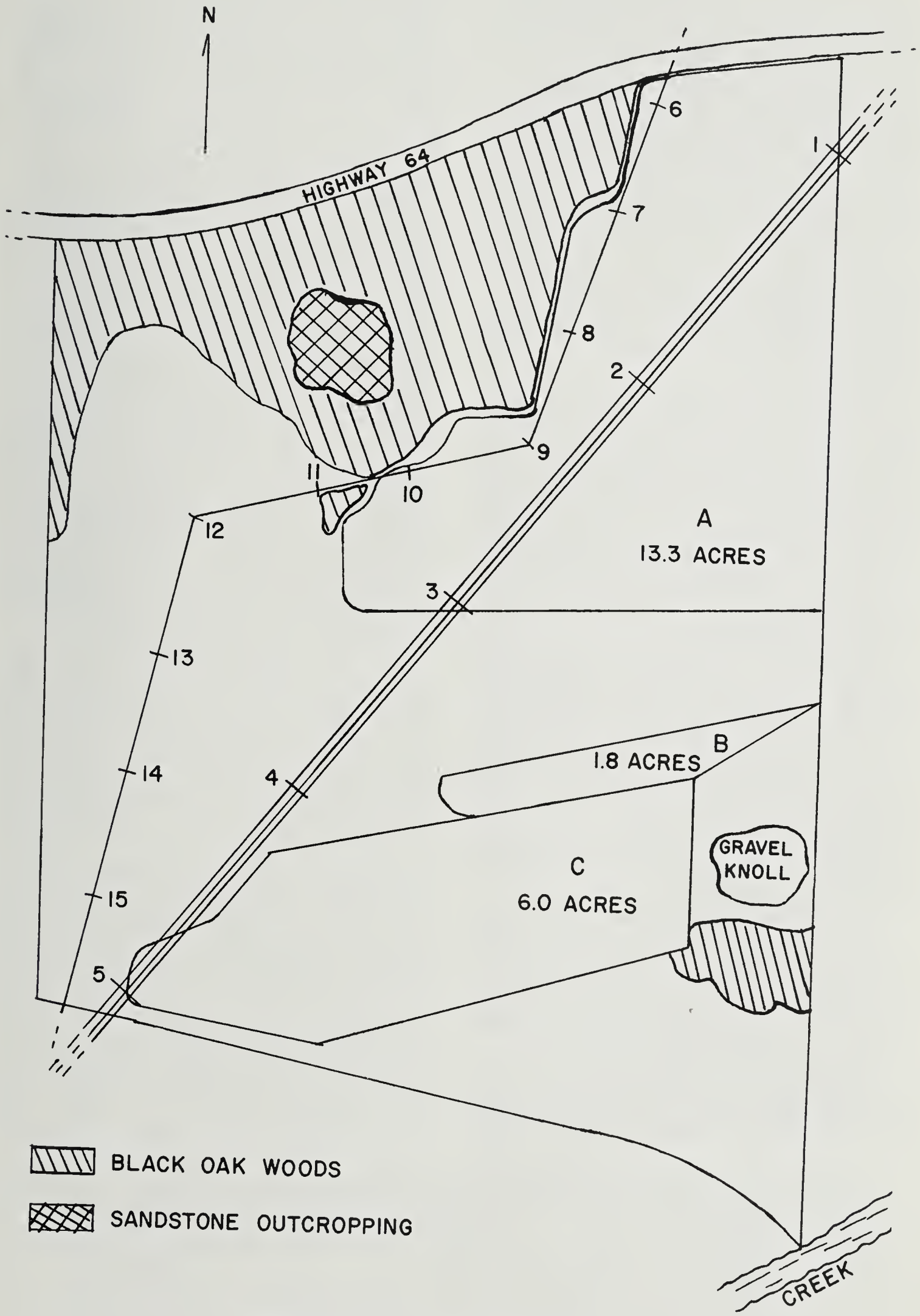

Figure 1.—Pine Rock Preserve.

area (except the lowest portion in the southwest corner) was plowed to the base of the sandstone rock outcroppings in the northwest corner. This took place in 1936. That year had been a dry year, as farmers in the area recollect, but the next year was too wet to allow planting of the field. Therefore, the existing prairie in the west part of the field has developed since 1936. About 1940 the 6.0 acre section (C) was planted to corn. Then Alonzo Miller bought the property in 1942 or 1943.

The lower triangle at the southeast was pastured in the late 1940's. Horses were pastured in this area, on the gravel knoll and among the adjacent oak trees during the 1950's. The creek at the southern tip of this triangle flooded frequently. This problem was later alleviated by dredging.

In 1963 and 1964 the land was rented from Miller by the Head family which has property adjacent to Pine Rock Preserve. Since the land did not promise much agricultural productivity, Richard Head marked off three portions of the preserve that had been previously cultivated and put them in the Soil Bank (see Table 1).

In 1964 the three soil bank areas were mowed for the last time. The property was sold to the Nature Conservancy in November of that year. It was deeded to Northern Illinois University in 1966.

These three sections of land which were most recently worked by Mr. Head are the major areas dealt with in this study. The Soil Conservation Service has used a letter coding system for indicating the different parcels of land. This same system will be maintained throughout this report. Section A to the northeast (see Figure 1) consists of 13.3 acres. Section C to the southeast consists of 6.0 acres and adjacent to C is a 1.8 acre strip of land designated B. Total area of these three pieces is 21.1 acres.

The soil in Pine Rock Preserve has not been mapped to our knowledge. However, the Soil Conservation Service has records of soils adjacent to the preserve. Sumner Sandy Loam appears to extend through part of section A and Houghton Muck between A and B. Most of A is composed of sand originating from the St. Peter sandstone outcrop to the west. It is this outcropping which is the origin of the name given to the preserve.

An obvious change which has taken place since the preserve was established is the extensive invasion of prairie grasses and forbs into the soil bank areas. The source of this invasion is the adjacent well-developed prairie. The invaders are mixed with remnants of farm weeds and introduced grasses and legumes.

Section A is very dry and sandy and is obviously a poor habitat for either herbaceous or woody plants. The area south of A has a lower elevation and is poorly drained by three small ditches to the south. It is our understanding that previous core borings revealed a tight clay pan subsoil which is at least par-

TABLE 1.—Soil Bank Crops at Pine Rock Preserve in 1963 and 1964.

Section	Acres	Crop
A	13.3	(1963) Oats (1964) Timothy
B	1.8	Red Clover
C	6.0	Red Clover
Combined	21.1	

tially responsible for the elevated water table. Section B had considerable standing water at the time of this study (October, 1970). The north, south and west sides of C were under water also.

Methods

It was decided that the most dependable data could be obtained from the preserve by identifying and mapping the location of each *woody* individual found in the three sections (see Figure 1) and under the electrical lines, both within the three sections and outside. Quadrats were established at each of the five double poles of the high line (poles 1 through 5 in Figure 1). Each quadrat was $10m^2$ with its center midway between the two poles. Transects 10m wide were drawn directly under the lines and extended between the quadrats at the double poles. Each specimen with its base in a transect or quadrat was included in the map of the sampling. All of the area under the high lines was mapped. Quadrats at single poles were $5m^2$ and transects between single poles were 5m wide. (Poles 6 through 15 in Figure 1.)

The remainder of the old field areas was mapped by using transects 10m wide which covered the entire surface of the three sections. Wooden stakes were used to mark off these strips which extended from one end of a section to the opposite end. As the length of the transect was walked each woody plant was identified and its approximate location in the field recorded. Therefore, any individuals not previously recorded under the electrical lines were included in these transects.

No plants were included if they were within 10m of a fence or concentration of larger trees constituting a fence-row or woods. Exact identification of the species of some specimens was difficult because many had lost their leaves at the time of the study late in October.

Observations and Results

The three sections of previously worked land show distinct differences in the woody plants which have invaded them. Each of the sections will be dealt with separately.

Section A. Only 109 specimens of woody plants (exclusive of *Rubus* sp.) were found in the 13.3 acres of Section A. The number of woody plants is very small for the size of the area because of the lack of moisture, the instability of the soil and the wide distribution of dewberries (*Rubus flagellaris* and *hispidus*) over much of the area. Erosion of the sandstone outcropping has been the source of the sand which predominates in this area and the resultant lack of a suitable environment for invaders is obvious. In a north central portion of A consisting of approximately $100m^2$ there were only two trees, both black oaks (*Quercus velutina*). This species is the dominant in the woods to the west (see Figure 1). A similar sparse population exists in the south central part of this section. A clearly different situation exists in the southwestern corner of A where approximately 10% of the total area contains nearly 38% of the total number of woody plants. Apparently the soil moisture conditions (the soil in this area is a sandy loam and the area has a lower elevation) explain the observed difference in density of the vegetation.

The following species were found in A but not in B or C: chokecherry (*Prunus virginiana*), multiflora rose (*Rosa multiflora*), sumac (*Rhus glabra*), grape (*Vitis riparia*), and prickly ash (*Zanthoxylum americanum*) (see Table 2). Quaking aspen (*Populus tremuloides*) occurs at

one place in this part of the preserve; it is invading by root sprouts from trees at the margin of the woods near pole 7 in the northwest corner (see Figure 1). A small clone of dogwood (*Cornus racemosa*) located in the southeastern part of A was not included in Table 2. This clone is not more than two feet in diameter and consists of 5 to 10 different stems.

Of the total individuals in section A 33% were dogwood (*Cornus stolonifera* and *racemosa*), 25% black cherry (*Prunus serotina*), 10% sumac (*Rhus glabra*), 8% maple (*Acer saccharinum*) and 6% elms (*Ulmus americana* and *rubra*) (see Table 2). Ten other species comprised the remaining 18%. It is obvious that a large proportion of the woody invaders was introduced by birds.

Section B. Section A is 7.5 times larger than B (see Figure 1), yet B had nearly the same number of woody individuals (109 for A vs. 94 for B). A had 16 species and 8 species were found in area B. The dominant species were similar in each section: dogwood and black cherry. However, the densities for each species varied. *Cornus* sp. made up 80% of the total in section B, while 5% were *Ulmus* sp. and 3% *Prunus serotina*. In addition, B was found to have 5% white ash (*Fraxinus americana*) (see Table 2).

Section B is low and poorly drained. Possibly as a result of this, the center of this thin strip of field is noticeably lacking in woody invaders. Dogwood (*C. racemosa*) seems to be able to tolerate this

TABLE 2.—Woody Plants of the Recently Cultivated Arears at Pine Rock Preserve

Species	Sect. A		Sect. B		Sect. C		Total
	No.	%	No.	%	No.	%	
Acer negundo	1	0.9	3	3.2	4	0.7	8
Acer saccharinum	9	8.2	1	1.1			10
Cornus stolonifera & racemosa	36	33.0	75	80.0	175	28.5	286
Fraxinus americana	3	2.8	5	5.3			8
Populus tremuloides					7	1.1	7
Prunus serotina	27	24.8	3	3.2	11	1.8	41
Prunus virginiana	2	1.8					2
Quercus velutina	4	3.7			9	1.5	13
Rhus glabra	11	10.2					11
Rhus radicans	1	0.9					1
Rosa multiflora	1	0.9					1
Rosa sp.	1	0.9			1	0.2	2
Rubus hispidus & flagellaris	many						
Salix sp.	4	3.7	1	1.1	19	3.1	24
Ulmus americana & rubra	7	6.4	5	5.3	388	63.2	400
Vaccinium sp.			1	1.1			1
Viburnum sp.					1	0.2	1
Vitis riparia	1	0.9					1
Zanthoxylum americanum	1	0.9					1
Total	109	100.0	94	100.0	615	100.0	818

Note: The numerous individual stems of extensive clones of *Rubus* were impossible to count and have not been included in the statistics of this table.

condition quite well. An old furrow, full of water at the time of the study, extends from east to west near the north side of B. This may form a ditch for partial drainage of the remainder of this area.

The east side of this section narrows to a pointed tip where there is a dense growth of herbaceous plants that apparently has not allowed woody invasion.

Section C. The north side of this section has not favored invasion by woody plants. Perhaps the seed source is too distant (which does not seem likely) or the soil conditions are not suitable for the available pioneer seeds. The south side of C, as noted for B, had a great deal of standing water during the course of the study. The middle of this old field is slightly higher and, consequently, dryer than the rest of C. Dogwoods (28%) and elms (*Ulmus americana* and *rubra*) (63%) make up the large majority of the 615 woody plants (Table 2). Other invaders in evidence were black cherry, black oak (from the small woods to the southeast) and seven quaking aspen. The quaking aspen have gained a foothold at the southeast corner and also at one spot half way across the south side of the field.

In the case of the elms a size gradient exists. Those located on the north side of the field are generally less than 1 foot in height while those on the south side may reach a height of 7 or 8 feet. The young elm seedlings of various sizes occur throughout the gradient. The accuracy of the count may be questioned at this point because the elms were so numerous in some areas that an estimation of the number was recorded. Two large elms (10 to 12 feet tall) were found in the row of trees and shrubs on the south side of C. These were *U. americana*. The closest *U. rubra* which could be seed sources for the area were located just outside the southwest corner of the preserve.

As noted earlier the south side of C is low and wet. Nineteen willows (*Salix* sp.) were located here. This is 3.1% of the total for C.

Invasion Associated with Electrical Lines

The electrical high lines cut through only one of the three old fields. This is section A. The poles for this high line are large double wooden poles which have two crossbars. The crossbars provide resting sites for birds. There are five wires in this high line which also may be used as perches. By analyzing the information gathered from this field it should be possible to compare the pattern of invasion associated with

Table 3.—Number of woody plants in 10m² quadrats at double poles (Pole sets numbered from north to south).

Species	Set 1	Set 2	Set 3	Set 4	Set 5	Total Numbers	Percent of Total
Cornus sp.	2	1	7	17	10	37	61.0
Morus sp.					1	1	1.6
Prunus serotina	1	7	2	3	3	16	26.0
Prunus sp.	3	1				4	6.6
Ulmus americana		1		1		2	3.3
Ulmus sp.					1	1	1.6
Total	6	10	9	21	15	61	100.1

the poles with that seen in the open area away from the poles and lines. Tables 3, 4 and 5 summarize this data. Only the first three sets of poles are located within section A. The species of invaders and frequency of their occurrence in the open area of section A (exclusive of the portion around the poles and under the wires) has been discussed earlier and is presented in Table 2. The same dominant species which were noted there (dogwood and black cherry) are found to be in the majority under the lines and around the poles. Fourteen of the 32 plants were dogwood and 11 were black cherry. Ten plants of each species were located at poles (Table 4).

Table 5 indicates the density of woody plants in different parts of section A. Previously it was pointed out that the southwest corner of this field has a high concentration of individuals, probably because of better soil and water conditions. Since this part of the field is not influenced by the high line, it is probably justifiable to subtract the area (about 7,270m^2) and the number of plants found in it (43). This leaves 80 plants in 65,300 m^2 which is 1/816m^2. This increases the relative density of plants under the lines (1/485m^2) when compared to the open field (1/816m^2 instead of 1/590m^2). Even without this manipulation of figures there is a definite concentration of woody plants at the poles (1/12m^2). Since it is possible for the seeds of all of the species except *Ulmus* to be transported by birds this would appear to support the hypothesis that lines and poles serve as resting sites for birds which deposit ingested seeds. Thus the presence of the power line promotes invasion of woody species.

A similar comparison was made in the area to the southwest of section A (Table 6). This part of the preserve which lies under the electrical line has not been under recent cultivation and is covered by a well-developed prairie sod. The density of plants at poles 4 and 5 is 1/5.4m^2 while in the area between pole quadrats it is only 1/79m^2. This information tends to further support the hypothesis that poles and to some extent lines, have been influential in the invasion of woody plants disseminated by birds. As in the case of the three soil bank areas, which have more woody plants in the southern part, there is a higher density of plants under the lines in the southern part of the preserve (1/79m^2 there as opposed to 1/485m^2 under the lines in section A). This reemphasizes the more favorable environment of the south part of the preserve.

A third example of concentration of plants at poles is illustrated in the southwest corner of the pre-

TABLE 4.—Number of woody plants at the three sets of poles (1, 2 and 3) and under the high lines in Section A.

Species	Combined 3 Pole Quadrats	Under Lines	Total
Acer negundo		1	1
Cornus (stolonifera and *racemosa*	10	4	14
Prunus serotina	10	1	11
Prunus sp.	4		4
Ulmus sp.	1	1	2
Total	25	7	32

TABLE 5.—Comparison of the number of woody plants in various parts of Section A.

Location	Area (m^2)	Number of Plants	Area per Plant (m^2)
Section A (excluding strip under the high line	72,570	123	590
Section A (excluding high line and southwest corner of A)	65,300	80	816
Area under high line (includes poles)	3,700	32	115
Area under high line	3,400	7	485
Area in pole quadrats	300	25	12

TABLE 6.—Density of woody plants under electrical high line outside of Section A, including poles 4 and 5.

Location	Area (m^2)	Number of Plants	Area per Plant (m^2)
Under the lines (excluding pole quadrats)	3,400	43	79
Pole quadrats (2)	200	37	5.4
Total	3,600	80	

serve. Under the lines between poles 13 and 15 (excluding the quadrat at pole 14) there are 4 black cherry trees. In the $5m^2$ quadrat at pole 14 there are 13 black cherry trees.

Table 7 summarizes the information gathered from single poles 6 through 9. The location of the poles can be determined by referring to Figure 1. Although this area is under the influence of the closely associated oak woods it would seem to be further evidence for the increased density at poles. There are 14 separate plants plus a clone of *Rhus glabra* located at the poles and only 11 plants in the much greater area between the poles. This table indicates how poor the soil is in this portion of the preserve. Pole 8 has no woody plants within 5m of its base. The area in the transect from pole 8 to pole 9 has only one woody invader (black oak). *Rhus glabra* was found as a very dense population at pole 9 and no attempt was made to determine the number of stems present. This may be another example of a species introduced by birds which later increased in number by vegetative means. Of the twelve species found in this part of the preserve 8 were probably introduced by birds.

The probable methods of introduction of the woody plants into the soil bank areas are indicated in Table 8. Of the 22 species identified, fourteen produce fruits commonly eaten by birds and perhaps by mammals. Ten of the fourteen are shrubs, two are vines, and two are trees. The eight remaining species of the 22 are trees, seven of which are commonly spread by the wind. One, the black oak, is often disseminated by squirrels.

DISCUSSION AND CONCLUSION

Pine Rock Preserve is an example of secondary succession on abandoned agricultural land. Former soil bank fields have been invaded by

TABLE 7.—Number of woody plants under the electrical line from pole #6 to pole #9

Species	Pole 6	Between 6-7	Pole 7	Between 7-8	Pole 8	Between 8-9	Pole 9	Total
Acer negundo (w)				1				1
Amalanchier sp.(b)	1							1
Cornus racemosa(b)							1	1
Gleditsia triacanthos (m)	1							1
Juniperus communis (b)				1				1
Populus tremuloides (w)		1						1
Prunus sp. (b)							1	1
Prunus serotina(b)	3	1		2			4	10
Prunus virginiana (b)			1					1
Rhus glabra (b)							many	many
Quercus velutina (m)			1	1		1		3
Vitus riparia (b)							1	1
Total	5	2	2	5	0	1	7+	22+

Note: Numerous stems of *Rhus glabra* have not been included in the totals.

Key: b, seeds commonly dispersed by birds
m, seeds commonly dispersed by mammals
w, seeds commonly dispersed by wind

various herbaceous prairie species and also by woody plants.

It was hypothesized that the electrical lines in the fields were influential in introducing woody species by providing resting sites for birds which had eaten the seeds. Although there is not direct evidence to support this there is indirect evidence in several situations that exist. When the density of the plants located at the poles is compared with the density under the lines there is nearly always a greater concentration at the poles. When these two values are compared with the density in the open field area there is a greater density of woody plants associated with the power lines.

It is conceivable that the land at the base of the poles did not receive the same treatment while it was being worked, for farm equipment could not have worked as efficiently near the poles. However, there were similarities between the information from both recently cultivated areas (the soil bank sections) and those parts of the field never under cultivation. In both cases there is a greater density of woody plants associated with the electrical lines. It is possible that some plants at the poles may have invaded prior to 1964 and, since they were located near the poles, were not disturbed by the farm equipment.

From the information gathered in this study it is obvious that woody plants have invaded the abandoned soil bank. If this area is to be developed and maintained as a prairie, efforts must be made to control this woody invasion and to prevent competition with prairie species by the multiplication and growth of trees and shrubs. At present, the most critical invasion is

that of dewberry which is spreading over large portions of section A.

It is evident that the woody invasion results from either windborne seeds or from introductions by birds and mammals. Under normal circumstances this invasion is impossible to prevent without management, except where the habitat is unsuitable or the ground surface is completely occupied by herbaceous plants.

In order to permit prairie development in the former soil bank areas without excessive competition from woody invaders, consideration should be given to various methods of control. Scattered trees and shrubs might be eliminated by the careful use of herbicides. More dense concentrations of woody plants, especially the *Cornus*, *Rhus*, and *Rubus*, could possibly be controlled by burning. Experimental and control plots need to be established soon, with various control methods used on a trial basis.

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Manuscript received June 18, 1971.

Table 8.—Methods of seed dispersal of woody plants in soil bank areas.

Species	Common Method of Seed Dispersal
Acer negundo	wind
Acer saccharinum	wind
Cornus racemosa	birds
Cornus stolonifera	birds
Fraxinus americana	wind
Populus tremuloides	wind
Prunus serotina	birds
Prunus virginiana	birds
Quercus velutina	mammals
Rhus glabra	birds
Rhus radicans	birds
Rosa multifora	birds
Rosa sp.	birds
Rubus flagellaris	birds
Rubus hispidus	birds
Salix sp.	wind
Ulmus americana	wind
Ulmus rubra	wind
Vaccinium sp.	birds
Viburnum sp.	birds
Vitis riparia	birds
Zanthoxylum americanum	birds

EFFECTS OF pH ON GROWTH OF *LEPTOSIRA OBOVATA* VISCHER (CHLOROPHYTA)

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ABSTRACT.—Ethanol extracts of *Leptosira obovata* were found to have absorbance peaks at 555 nm and 685 nm. Absorbance was used as a measure of growth. Initial pH values of 3.0 and 8.0 completely inhibited growth, whereas 5.0 was optimal. pH declined during the first two days of culturing and then stabilized at 6.7, generally by the 6th day.

Many effects of pH on algal activities have been investigated, including effects on growth (Osterlind, 1950), photosynthesis (Emerson and Green, 1951), respiration (Reazin, 1954), availability of inorganic nutrients (Myers, 1962), permeability of cell membranes to water (Seemann, 1950), uptake of sugar (Taylor, 1950), uptake of IAA (Albaum, *et al.*, 1937), uptake of 2,4-D (Wedding and Erickson, 1957), asexual reproduction *via* zoospores (Ulehla, 1933), and sexual reproduction (Coleman, 1962). Since these studies included many species representing various taxa, different pH values, and different nutrient media, comprehensive generalization regarding effects of pH does not seem feasible. Rather, it is probable that a different pH range and optimum exists for each species-environment combination. This study documents the reaction of *Leptosira* to pH as a basis for further physiological and cultural studies.

MATERIALS AND METHODS

Leptosira obovata Vischer was obtained in axenic culture from the culture collection of algae at Indiana University. The culture medium was a modification of Bristol's Medium (Kulfinski and Henschel, 1969) supplemented with 2.5 g/l glucose. Fifteen ml of medium were placed in each 18 x 150 mm test tube, after which the tubes were stoppered with color-coded "Eonoplugs" and autoclaved for 20 min at 15 psi and 250° F. Each tube was inoculated with a 2 mm diameter loopfull of agitated matured inoculum.

Inoculated tubes were randomized in racks which were placed before cool-white fluorescent lights at an intensity of 500 foot-candles in cycles of 16 hours light and 8 hours darkness. Room temperature was maintained at 23° C and culturing time was 14 days.

Cultures which had been grown for 14 days in unaltered medium were collected by filtration. The filter paper and cells were extracted in 10 ml 95% ethanol for 24 hours at 23° C and then refiltered. The absorption spectrum (Table 1) of the extract was determined using a colorimeter. A broad absorption peak was found at 555 nm and a sharp peak at 685 nm. Both wavelengths were employed for absorbance measurements throughout the study. Absorbance is proportional to quantity of pigment and, therefore, to quantity of cells.

The pH of the unaltered medium was determined to be 6.7. In the growth experiment, pH was adjusted to 3.0, 4.0, 5.0, and 6.0 using 0.1N HC1 and to 7.0 and 8.0 using 0.1N NaOH. Twenty-five "trials" of this experiment were performed, each one representing a different batch of medium and of inoculum. Each trial contained five replicates,

TABLE 1.—Absorbtion spectrum of an ethanol extract of the alga *Leptosira obovata*. Two absorbance peaks exist, one at 555 nm and one at 685 nm.

nm	Absorbance	nm	Absorbance
450	0.039	625	0.229
475	0.100	650	0.125
500	0.342	675	0.194
525	0.629	685	0.305
550	0.668	700	0.215
555	0.675	725	0.097
575	0.561	750	0.056
600	0.415		

giving 125 absorbance readings per pH value per wavelength.

RESULTS AND CONCLUSIONS

Effects of pH on growth.—The greatest amount of growth was found at pH 5.0 and the second greatest at pH 6.7 (Table 2). A broad pH range, from 4.0 through 7.0, permitted growth, but pH 3.0 and pH 8.0 were completely inhibitory.

The two wavelengths employed gave generally consistent results, that is, the pH values could be ranked identically in absorbance at either wavelength. At 685 nm, the absorbance resulting from pH 5.0 was 1.4x that from 4.0, whereas at 555 nm, the absorbance from pH 5.0 was 2x that from pH 4.0. We suggest that this difference in degree of absorbance is the result of the extract containing several pigments, with the pigments contributing differentially to absorbance at a given wavelength.

It should be pointed out that the absorbance at pH 6.0 was significantly less than that for either 5.0 or 6.7, whereas one might expect it to be equal to or greater than either of these. Actually, pH 6.0 yielded a lower absorbance than the adjacent pH's in 20 out of 25 trials and was greater than both in only one of 25 trials. We conclude, therefore, that this is a real effect, rather than an artifact. We suggest that this effect is probably the result of a pH-nutrient absorption interaction.

Changes in pH with Time.—Cultures were incubated for 14 days and were pH sampled at two day intervals. With initial pH's adjusted to 5.0, 6.0, 6.7, and 7.0, there was an initial decline of pH of all but 5.0 followed by an increase to a plateau at 6.7 (Table 3). The changes in pH may be attributable to differential ion absorption and release. Spent and fresh media were titrated with HC1 and NaOH. The spent medium and the fresh were virtually identical in response to added acid and alkali with the exception that the spent was slightly more acidic. Both media exhibited plateaus at pH 2.0 and at pH 12.0.

TABLE 2.—Absorbance by pigment extracts in relation to initial pH of the culture medium. Each number is the mean of 5 replicates (test tubes) per trial times 25 trials, each trial representing a different batch of medium, a different period of time, and a different batch of inoculum.

Wavelength	Initial pH						
	3.0	4.0	5.0	6.0	6.7	7.0	8.0
555 nm	0.000	0.064	0.123	0.075	0.098	0.088	0.000
685 nm	0.000	0.219	0.296	0.237	0.260	0.247	0.000

TABLE 3.—Changes in the pH of the nutrient medium with time. The unadjusted medium had a pH of 6.7. Those with lower initial pH were HC1 adjusted and the one at pH 7.0 was NaOH adjusted.

Elapsed Time	Initial pH			
	5.0	6.0	6.7	7.0
2 days	5.4	5.8	6.1	6.2
4 days	6.1	6.2	6.5	6.4
6 days	6.7	6.6	6.7	6.7
8 days	6.7	6.7	6.7	6.7
10 days	6.7	6.7	6.7	6.7
12 days	6.7	6.7	6.7	6.7
14 days	6.7	6.7	6.7	6.7

Therefore, the alga did not stabilize pH at 6.7 by secretion of buffering agents and it must be concluded that maintenance of this pH level was the result of an ionic equilibrium between algal cells and medium.

SUMMARY

1. An absorption spectrum was obtained with absorbance peaks at 555 nm and at 685 nm. While the absorbance measurements at these wavelengths were different in magnitude, they were consistent with one another.

2. Initial pH's of 3.0 and 8.0 prevented growth.

3. Maximum growth occurred in response to an initial pH of 5.0.

4. Media generally exhibited an initial decline in pH followed by a stabilization at pH 6.7, which was, coincidentally, the pH of the unaltered medium.

5. Titration with acid or alkali produced comparable pH changes between fresh and spent media, with no indication of additional buffering in the latter.

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Manuscript received July 28, 1971.

ADDITIONS TO THE FLORA OF EDGAR COUNTY, ILLINOIS

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ABSTRACT.—210 species of vascular plants in 72 families were collected in Baber Woods. Of the plants collected, 40 were new records for Edgar County. The species are listed with collection numbers.

In an ecological survey of the vegetation of Baber Woods, a number of plants were found that have not been previously recorded for Edgar County, Illinois. This woods, a natural area owned by the Nature Conservancy, is located in the NW1/4 of Section 18, T12N, R13W, Edgar County, Illinois. It is on a part of the Shelbyville Moraine which traverses this section of Illinois in an east-west direction and represents the southern extent of the Wisconsin glaciation. This woods is a remnant of a once larger forest on this moraine.

The Baber family purchased the first portion of the timber in 1835 and by 1894 had acquired more than 61 acres. Due to a road built in 1900, 10 acres of the original tract lies separated to the west and has been cleared of trees. Since 1900, the woods has been disturbed very little. Previous to 1900, however, a few trees were removed for firewood and fences and some herbs (ginseng) were removed. The woods has never been completely cut except for a three acre lot in the southwest corner. At this site once stood a one-room cabin and a small log barn. According to Mr. Adin Baber, these were not in use in 1903, and the buildings were hauled away for firewood. The woods has never been pastured during the Baber ownership.

A complete survey of the woody vegetation of Baber Woods was conducted by McClain and Ebinger (1968). In that survey, three distinct woody vegetation types were recognized. These vegetation types are: a white oak-sugar maple dominated region in the west and northwest parts of the woods, a disturbed region in the southwest corner where the cabin and barn once stood, and the remainder of the woods which is dominated by species of oaks and hickories.

During the present study, a total of 210 species of vascular plants in 72 families was collected in the woodlot. Of these, 9 were fern or fern-allies, 43 were monocots, and 158 were dicots. Of this latter group, 49 were trees, shrubs, or woody vines, while 109 were herbs. The largest family represented was the Compositae with 22 taxa, followed by the Gramineae with 15. Of the plants collected, 40 were new records for Edgar County, according to Jones and Fuller (1955) and Winterringer and Evers (1960). These new records encountered, with the collector, collecting number, and general distribution in the woods are listed below. All specimens are deposited in the Stover Herbarium of Eastern Illinois University.

OPHIOGLOSSACEAE

Botrychium dissectum Spreng. Partial shade in disturbed area; Ebinger 7925.

Botrychium obliquum Muhl. Partial shade in disturbed area; Ebinger 7926.

Ophioglossum vulgatum L. Full sun in disturbed area; Kloker 39.

CYPERACEAE

Carex hirsutella Mack. Partial shade in disturbed area; Kloker 227.

Carex jamesii Schw. Southern, open part of oak-hickory area; Kloker 228.

Carex sparganioides Muhl. Disturbed area and southern, open part of oak-hickory area; Ebinger 7668.

GRAMINEAE

Leersia virginica Willd. Moist depressions in the oak-hickory area; Ebinger 7893.

Paspalum pubescens Muhl. Edge of woods; Ebinger 6037.

Poa compressa L. Disturbed area and open parts of oak-hickory area; Kloker 244.

JUNCACEAE

Juncus interior Wieg. Full sun in disturbed area; Ebinger 7666.

LILIACEAE

Hemerocallis fulva L. Edge of woods; Ebinger 7612.

Ornithogalum umbellatum L. Partial shade in disturbed area; Ebinger 5328.

ORCHIDACEAE

Corallorhiza odontorhiza (Willd.) Nutt. Partial shade in disturbed area; Ebinger 6621.

Corallorhiza wisteriana Conrad. Partial shade in disturbed area; Kloker 50.

Liparis lilifolia (L.) Rich. Partial shade in disturbed area; Kloker 251.

ACERACEAE

Acer saccharinum L. Disturbed area; Ebinger 7950.

ARALIACEAE

Panax quinquefolius L. Densely shaded part of oak-hickory area; Kloker 311.

ASCLEPIADACEAE

Asclepias exaltata (L.) Muhl. Shaded part of oak-hickory area; Kloker 87.

CAMPANULACEAE

Specularia perfoliata (L.) A. DC. Edge of road and oak-maple area; Kloker 250.

CAPRIFOLIACEAE

Viburnum recognitum Fern. In openings of the oak-hickory area; Ebinger 7670.

CARYOPHYLLACEAE

Stellaria media (L.) Vill. Full sun in disturbed area; Kloker 65.

COMPOSITAE

Aster lateriflorus (L.) Britt. Opening in oak-hickory area; Ebinger 7920.

Aster sagittifolius Wedem. Southern, open part of oak-hickory area; Kloker 232.

Aster shortii Lindl. Southern, open part of oak-hickory area; Ebinger 7922.

Prenanthes altissima L. Southern, open part of oak-hickory area; Ebinger 7924.

Solidago caesia L. Throughout open parts of woods; Ebinger 7921.

Solidago nemoralis Ait. Southern, open part of oak-hickory area; Kloker 219.

EBENACEAE

Diospyros virginiana L. Disturbed area; Ebinger 6039.

ERICACEAE

Monotropa lanuginosa Michx. Upland, shaded part of oak-maple area; Ebinger 7672.

Monotropa uniflora L. Upland, shaded part of oak-maple area; Ebinger 6044.

EUPHORBIACEAE

Acalypha virginica L. Full sun in disturbed area; Ebinger 7923.

HYDROPHYLLACEAE

Ellisia nyctelea L. Shaded parts of oak-hickory area; Kloker 223.

JUGLANDACEAE

Carya glabra (Mill.) Sweet. Throughout woods; Ebinger 6041.

LABIATAE

Monarda fistulosa L. Full sun in disturbed area; Ebinger 5795.

LEGUMINOSAE

Amphicarpa bracteata (L.) Fern. Vine in shaded parts of oak-hickory and oak-maple area; Ebinger 7892.

Desmodium paniculatum (L.) DC. Full sun in disturbed area; Ebinger 7900.

POLYGONACEAE

Rumex crispus L. Partial shade in disturbed area; Kloker 94.

Rumex obtusifolius L. Moist depressions in the oak-hickory area; Ebinger 7966.

ROSACEAE

Rubus occidentalis L. Disturbed area; Ebinger 7957.

SOLANACEAE

Physalis heterophylla Nees. Partial shade in disturbed area; Kloker 257.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Robert A. Evers, Illinois Natural History Survey, for his help in identifying the plants collected during this study.

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Manuscript received November 12, 1971.

THE IMMATURE STAGES OF *CHIRONOMUS AETHIOPS* (TOWNES) WITH KEYS TO THE SPECIES OF THE KNOWN IMMATURE STAGES OF THE SUBGENUS *DICROTENDIPES* (CHIRONOMIDAE: DIPTERA)

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ABSTRACT.—The immature stages of *Chironomus* (*Dicrotendipes*) *aethiops* (Townes) are described and an extension of its North American range is noted. A key to the immature stages of known North American species is included.

Chironomus (*Dicrotendipes*) *aethiops* (=*Tendipes* (*Limnochironomus*) *aethiops*) was described by Townes (1945) from specimens collected in New Mexico. It has subsequently been recorded by Beck and Beck (1959) from the coastal lowlands and central highlands of Florida. The material presented in this paper was collected by D. Erman, during a limnological study of the littoral zone of Bear Lake, Rich County, Utah. Mr. Erman was able to provide adult specimens as well as numerous live larvae living in *Scirpus acutus* Muhlenberg from which twelve larvae were reared through to the adult.

Chironomus (*Dicrotendipes*) *aethiops* (Townes)

Pupa:

Length 5.6 ± 0.19 mm (N=8). Cephalic tubercle (Fig. 1) 1.5 times as wide as long, subapical setae 0.5 times as long as tubercle. Respiratory apparatus filamentous, arising from 2 main stems. Tergite 1 (Fig. 2) with 2 small anterio-lateral patches of spinnules and without posterior band of spines. Tergites 2-6 with broad mesal patch of spinnules, becoming coarser towards posterior of segment; tergite 2 with single transverse row of anteriorly directed hooks across posterior margin; tergite 4 and 5 with small caudolateral patch of spines. Tergite 7 with narrow transverse band of spinnules across anterior fourth of segment. Tergite 8 with bifid caudolateral spur (Fig. 3). Tergites 5-8 with 4 lateral filamentous setae, respectively. Tergite 9 with 48 ± 2 (N=15) natatory setae.

Larva:

Length 7.8 ± 0.34 mm (N=4). Color blood red with mottled green pigmentation covering thoracic segments. Head pale yellow, 1.3-1.4 times as long as wide; eyespots black. Antenna (Fig. 4) pale yellow, 5 segments (36:10:6:6:2), basal segment 3 times as long as wide, sensorium 0.31 of way from base; antennal blade attached to base of second segment, extending beyond tip of apical segment; single broad lauterborne organ present at base of third segment, extending to middle of third segment. Mandible (Fig. 5) pale yellow, 1.6 times as long as wide, apical tooth and 3 lateral teeth black; dorsomesal tooth paler, not extending beyond apical tooth; accessory tooth (Fig. 6) knife shaped, extends to apex of third lateral tooth; preapical setae present; caudolateral brush arising from 3 distinct stems. Epipharyngeal plate with 5 teeth (Fig. 7). Labial plate (Fig. 8) blackish brown; 1.5 times as wide as long; medial tooth simple; first laterals equal in height or slightly longer than medial tooth; second laterals small, basal 5/6th fused to first laterals; remaining 4 laterals descending in

height. Paralabial plates (Fig. 8) pale yellow, striated, widely separate medially, 1.7 times wider than long, anterior margin crenulate. Premandibles (Fig. 9) bifid, inner blade broader than outer. Anterior prolegs separated medially. Anal papillae short, 1.9 times as wide as long, each with 8 coarse apical setae and 2 fine basal setae. Two pairs of short, obtuse anal gills.

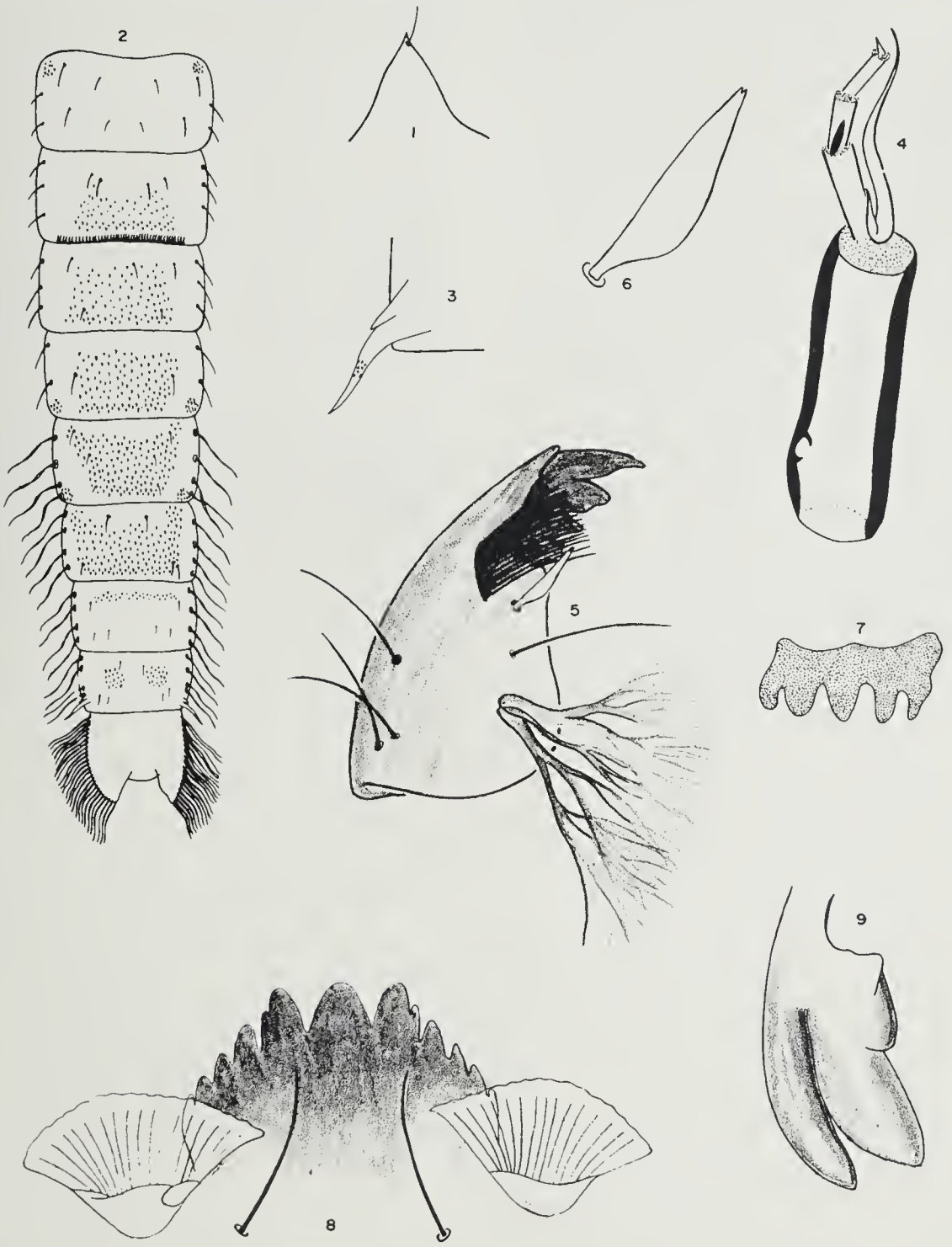

FIGURE 1. *C.* (*D.*) *aethiops*, cephalic tubercle of pupa.
FIGURE 2.—dorsal view of abdominal tergites of pupa.
FIGURE 3.—dorsal view of left caudolateral spur on abdominal segment 8 of pupa.
FIGURE 4.—larval antenna.
FIGURE 5.—ventral view of larval mandible.
FIGURE 6.—accessory tooth of larval mandible.
FIGURE 7.—larval epipharyngeal plate.
FIGURE 8.—ventral view of labial plate of larva.
FIGURE 9.—ventral view of premandible of larva.

Key to the Known Larvae of *Chironomus* (*Dicrotendipes*)

1. First antennal segment about 2.2 times length of second segment.....*nervosus* Staeger
   First antennal segment greater than 2.8 times length of second segment...2
2. First lateral teeth of labial plate barely notched.....*neomodestus* Malloch
   First lateral teeth of labial plate deeply notched.....3
3. Inner and outer blades of premandibles equal in width.....*fumidus* Johannsen
   Inner blade of premandible at least twice as wide as outer blade.....4
4. Anterior margin of paralabial plate smooth.....*californicus* Johannsen
   Anterior margin of paralabial plate crenulate.....*aethiops* (Townes)
   modestus Say

Key to the Known Pupae of *Chironomus* (*Dicrotendipes*)

1. Caudolateral spur on eighth abdominal tergite bifid.....2
   Caudolateral spur on eighth abdominal tergite simple.....3
2. First abdominal tergite without posterior band of spines.....*aethiops* (Townes)
   First abdominal tergite with posterior band of spines.....*nervosus* Staeger
3. Apical abdominal tergite with less than 50 anal filaments.....*neomodestus* Malloch
   Apical abdominal tergite with 50 or more anal filaments.....4
4. First abdominal tergite with anteriolateral patch of spines.....*modestus* Say
   First abdominal tergite without anteriorlateral patch of spines.....*californicus* Johannsen

Acknowledgments

The author wishes to express his gratitude to D. Erman for his cooperation in collecting live material for purposes of rearing and to H. K. Townes, Jr. for the loan of paratypes to verify the identification.

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Manuscript received October 11, 1971.

NOTES

FIRST ILLINOIS SPECIMEN OF THE ROCK WREN

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A rock wren (*Salpinctes obsoletus*) from Normal, McLean County, represents the first recorded specimen for Illinois. It was found November 2, 1970 near the entrance to the Department of Biology greenhouse on the Illinois State University campus by an unidentified student and brought to us. The specimen, now in the I.S.U. collection, was prepared by the freeze-dry method and was not sexed.

The only other record of this species for the state is a sighting May 30, 1926 at Urbana, Champaign County (Hyde, 1927). Other extralimital localities from which specimens or sightings have been reported include Michigan (Van Tyne, 1942), Tennessee (Newman, 1957), Alabama (James, 1965), Massachusetts (Christie and Emory, 1966), and Ontario (Burger and Brownstein, 1967).

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Van Tyne, J. 1942. A rock wren specimen from Michigan. Wilson Bull. 54:52.

Manuscript received July 28, 1971.

Transactions Illinois Academy of Science

PREPARATION AND THERMAL STABILITY OF BIS (4-CYANOPYRIDINE) PALLADIUM (II) CHLORIDE

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Recently, the complexes of several metals with 4-cyanopyridine (4-CP) (Farah and Iwamoto, 1965) and 4-cyanopyridine N-oxide (Nathan, et al., 1969; Piovesana and Selbin, 1969) have been described. The complexes with these ligands were studied to determine the mode of coordination of the ligand to the metal ion and to see if these ligands form bridged complexes. No evidence was found for nitrile coordination in complexes of 4-CP but nitrile coordination was found to occur in 2-CP and 3-CP (Farah and Iwamoto, 1965).

The solid complex of 4-CP with silver nitrate has been found to have much higher thermal stability than similar complexes containing some other pyridines which are considerably more basic (Akhavein and House, 1970); however, this complex involves coordination only through the pyridine nitrogen atom (Farah and Iwamoto, 1965; House and Akhavein, 1970). We have, therefore, been interested in the donor properties of the nitrile nitrogen in 4-CP.

We have found that heating produces stepwise decomposition of solid complexes of palladium (II) chloride which contain ligands having two donor atoms of about equal strength (House and Adams, 1970). This report describes the results of thermal studies on bis (4-cyanopyridine) palladium (II) chloride, which contains donor atoms of greatly different strengths.

Experimental

A solution of 4-CP was prepared containing 0.48g (5.0 m mole) in 25ml of absolute ethanol. To this solution was added 2.5 m mole of $PdCl_2$. The mixture was refluxed for several hours and yellow solid was obtained. The precipitate was separated by filtration, washed with acetone, and then air dried. Weight loss studies show that the complex contains two ligands per palladium.

Similar procedures using 4-cyanopyridine N-oxide gave dark brown ill-defined products, which were not studied further.

Thermal measurements were performed as previously described (House and Adams, 1970) using a Perkin-Elmer Differential Scanning Calorimeter, Model DSC-1B.

Results and Discussion

The thermograms of $Pd(4\text{-}CP)_2Cl_2$ show only one endotherm beginning at 303°C. The samples were held at 377°C for 15 minutes after scanning to that temperature. Results obtained from several thermograms are shown in Table 1.

Table 1.—Thermal data for decomposition of $Pd(4\text{-}CP)_2Cl_2$.

Initiation Temp., °C.	% Mass loss* Calcd	Found**	△H,** kcal/mole	Ea** kcal/mole
303	54.6	53.7±2.6	19.4±2.5	60.2±1.0

*Mass loss for loss of two molecules of 4-CP per molecule of complex.

**Shown as average value ± average deviation from the mean.

Unlike the pyrazine complexes which show stepwise decomposition (House and Adams, 1970), no evidence has been found for stepwise decomposition, which would presumably involve bridging of the ligands after loss of one molecule of ligand.

When the two nitrogen atoms in 4-CP are involved in hydrogen bond formation with methyl alcohol, the nitrile nitrogen and the pyridine nitrogen cause the OH stretching band to be shifted by 61 and 212 cm^{-1} respectively from its "free" position of 3641 cm^{-1} (Cook and House, 1969). Thus, the donor properties of the nitrile nitrogen are not nearly as great as those of the pyridine nitrogen. Coordination by the nitrile group appears to be too weak to cause the 1:1 complex involving coordination by both nitrogen atoms to be stable as a decomposition product. Coordination of a nitrile group in the 4-position thus appears to be much weaker than previously believed and insignificant compared to that of the pyridine ring. This is in contrast to the behavior of 2- and 3-cyanopyridine (Farah and Iwamoto, 1965).

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Manuscript received May 18, 1971.

Transactions Illinois Academy of Science

THE FRESH-WATER JELLYFISH, CRASPEDACUSTA SOWERBYI IN ILLINOIS

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ABSTRACT:—Distribution of *Craspedacusta sowerbyi* in Illinois, with extensions of the range into the northern portion of the state.

The fresh-water Jellyfish (*Craspedacusta sowerbyi*) is widely distributed throughout the world. Kramp (1950) and Sowerby (1941) present evidence relating to the origin within the Yangtze River valley in China. The dissemination of this species throughout the world is attributed to shipments of aquatic plants from China.

Summaries of the geographical distribution and seasonal occurrence of this species within the United States are available in papers by Dexter et al. (1949), Lytle (1960, 1962) and Pennak (1956). The medusa has been reported from 29 states and the District of Columbia in the United States. In some states, Ohio, Pennsylvania, and Michigan, *Craspedacusta* has been taken from numerous localities, but the remaining states only one to several localities are established.

Craspedacusta sowerbyi has been reported predominately from fish ponds, quarries, reservoirs and abandoned gravel pits. This tends to limit the distribution through natural drainage systems.

In recent years the occurrence of *C. sowerbyi* in streams has become more frequent. Garman (1916) first reported medusae from a creek in Kentucky; Baird (1932) from a river in Ohio; Dexter (1949) from a stream in Pennsylvania, while Bushnell and Porter (1967) added thir teen new distributional records for the state of Michigan. Three of these localities; Swan Creek, Allegan Co.; Augusta Creek, Kalamazoo Co., and Red Cedar River, Ingham Co., add additional stream habitats.

In Illinois and Wisconsin the distributional pattern and habitat are poorly known. Illinois is represented by only five localities, extending into the central portion of the state, while Wisconsin lacks recoveries.

Van Cleave (1936) first reported the medusae within the state, from Vandalia, Fayette County, and Carmi, White County. Schmitt (1939) cites another visual record from Vandalia,, while Steagall (1937) records a medusa taken from the State water tank at Anna, Union Co., Illinois.

Moore (1954) summarized the distribution of *C. sowerbyi* within Illinois, and added two new localities, and the first for central Illinois. All previous stations having been from the southern portion of the state. On 1 September 1952, Moore collected five specimens from Sunset Pond, one and one-half miles southeast of Oakwood, Vermilion Co., Illinois, and on 5 September 1953 a large number were observed (thirteen collected) at Fairmount, Vermilion Co., Illinois. Both of

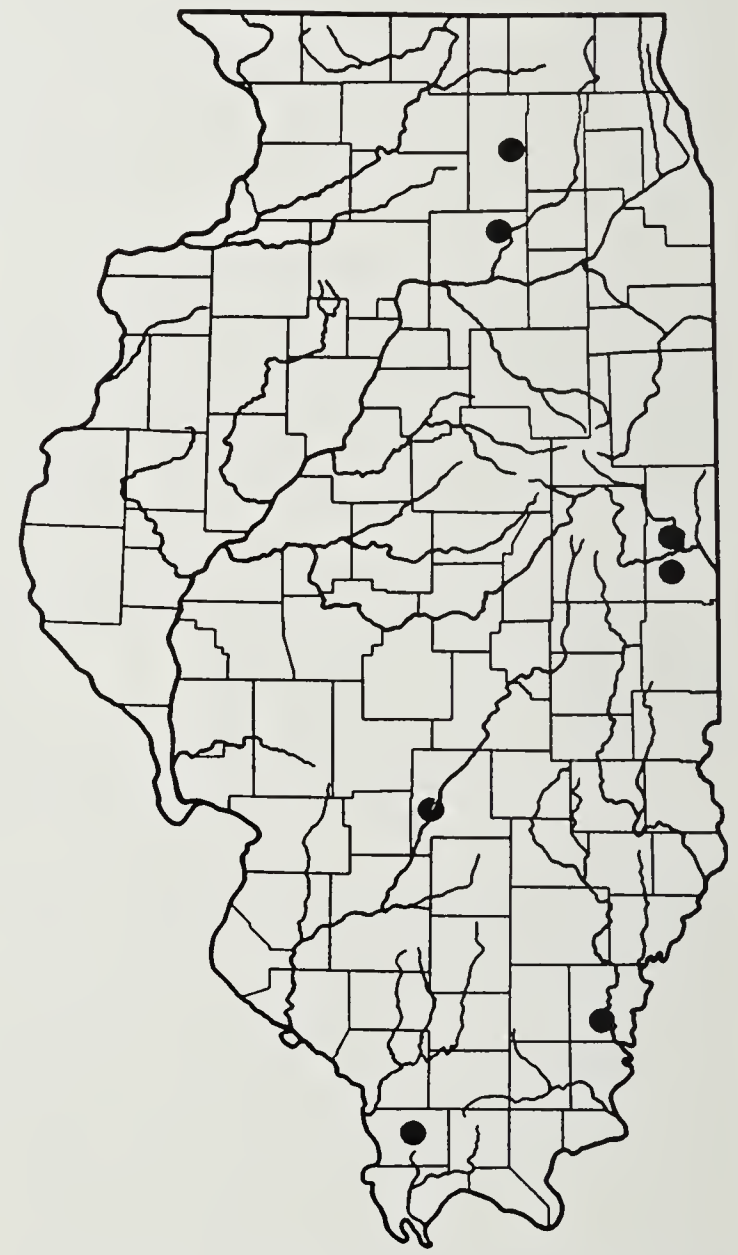

FIGURE 1.—Records of *Craspedacusta sowerbyi*.

these localities are from strip-mining quarries.

The present report adds two additional localities, and the first records for northern Illinois (Figure 1).

On the evening of August 22, 1966, *C. sowerbyi* was first noticed by Mr. Ivan Sams of Sandwich, in the Sheridan Rod & Gun Club quarry, one-half mile north of Sheridan, LaSalle County, Illinois. Mr. Sams stated "thousands of specimens were visible on the surface of lake", and twenty were collected and brought to me for identification. All the specimens were females, measuring no less than 15-25 mm.

At 1800 hour, 15 September 1966 we revisited the quarry, and collected fifty-five additional specimens. The water was clear and calm, with medusa so abundant at the surface, that it was almost impossible to miss collecting specimens with a dip-net. The medusae seemed to congregate near shore, and were noted to slowly surface, turn over and float downward.

The Sheridan Rod & Gun Club lake is an abandoned gravel quarry, no less than 50 years old, covering 40 acres. The average depth is 12 feet, with a bottom consisting of sand covered with silt. The only aquatic plants noted were along the north and west margins of the quarry, and consisted of *Nitella* sp., *Equisetum fluviatile, Ranunculus trichophyllus, Nuphar advena, Sagittaria latifolia*, and *Potamogeton* species.

On September 5, 1966, twelve medusae were taken from a small gravel quarry, 5 miles southwest of DeKalb, DeKalb County, Illinois. Only a few specimens were noted at this site, and collecting was limited due to the abrupt drop-off near shore.

Additional specimens were collected on 12 September 1967 at the LaSalle County station, although since that time I have been unable to relocate this species in northern Illinois.

Specimens are deposited in my private collection, and the Northern Illinois University Invertebrate Collection, under Dr. C. K. Mathers.

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Manuscript received June 10, 1971.

REVEREND ROBERT ROLLAND BRINKER
1905-1970

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The Reverend Robert R. Brinker, O.F.M., professor of biological sciences at Quincy College and Second Vice-President of the Illinois State Academy of Science in 1959-1960, died of a heart seizure April 2, 1970, near St. Louis, Missouri. Father Robert, whose body was found in his automobile, was apparently returning to Quincy after a few days visit in St. Louis.

Robert R. Brinker was born October 15, 1905, in St. Louis, Missouri, the son of Frederick and Irene Gausepohl Brinker. As a boy he attended St. George, an elementary school in St. Louis. The family moved to Richmond, Indiana, where he attended St. Andrews, and when the family moved to Indianapolis, Indiana, he spent three years at St. Catherine.

Father Robert R. Brinker in 1967.

Father Robert entered the Franciscan Order at Teutopolis, Illinois, on July 1, 1925, and was ordained to the priesthood on May 5, 1932, at Indianapolis. After his ordination, he spent a year in the study of sacred eloquence at St. Louis. Later, he was parish assistant and chaplain of St. Joseph Hospital at Ashland, Wisconsin. In 1936, he came to Quincy, Illinois, where he served the Quincy College Academy for a short time as a science teacher and later taught botany in the biology department at Quincy, College.

From 1940-1942, Father Robert pursued graduate studies in botany at the Henry Shaw School of Botany, Washington University, St. Louis, and received a master of science degree. He described eight new species of *Schoenocaulon* in a paper entitled, "Monograph of the Genus Schoenocaulon" published in the Annals of the Missouri Botanical Garden 29(4): 287-314, 1942.

In 1942, Father Robert returned to Quincy College to resume teaching in the biological sciences, and was concurrently an instructor at St. Marys Hospital School of Nursing. At Quincy College, Father Robert was chairman of the division of natural sciences and chairman of the department of biological sciences until 1960. He was also a member of the Sigma Chi Honors Society.

During his stay at Quincy College, Father Robert attained a wide reputation as a landscape gardener and as a collector of the flora of Adams County, Illinois. His flowering plant herbarium containing approximately 1,850 specimens is preserved in the herbarium of the Illinois Natural History Survey, Urbana, Illinois. He was interested in the study of mosses and published three papers in 1948. These were "Plagiochila Dotensis." Bryologist 51(1):1-4; "Plagiochila Standleyi." Bryologist 51(1):4-6; and "Plagiochila tricarinata." Bryologist 51(1):7-9. His moss collection is in the herbarium of the Missouri Botanical Garden.

In 1960, after he performed the duties of Second Vice-President of the Academy excellently and efficiently, Father Robert was transferred to St. James Trade School at Riverton, Illinois, where he served as chaplain and instructor. He returned to Quincy College in 1963 as professor of biological sciences, a post he held until his death.

Father Robert was not the ideal lecturer, but he was an excellent teacher. His main objective was to help his students learn. He believed that learning does not consist merely in memorizing but that knowledge also comes from doing the things about which the student reads and hears. A former student in systematic botany supplied a striking example, "I began to realize some of the problems a taxonomist faces when Father Robert assigned us the task of constructing a key to the oaks of Adams County. One cannot construct a key unless he knows something about the organisms included in the key. From one assignment, therefore, I learned something about oaks, about the problems of a taxonomist, and about the construction of keys."

Father Robert was equally at home in the classroom or in the kitchen. He enjoyed gourmet cooking, a task at which he attained much notoriety upon the completion of his masterpiece, the roast suckling pig. He was chief cook on summer school trips to Florida and the Southwest and would work equally hard to produce a pot of fish soup, broiled lobsters from the Gulf of Mexico, or a sauce for the biology club pizza party.

Over the years Father Robert proved to be deeply committed to the welfare of his community. He served as medical field agent for Adams County and spent many Christmas and Easter Holidays assisting in various parishes of the diocese.

To those of us who knew Father Robert, he was a kind and sympathetic man with an excellent sense of humor. Let us hope that the world sees more of his type.

Manuscript received May 28, 1971.

Transactions Illinois Academy of Science

CULTURAL CHARACTERISTICS OF SOME ILLINOIS RESUPINATE BASIDIOMYCETES

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ABSTRACT.—The cultural characteristics of three resupinate Basidiomycetes are reported for the first time.

In recent years taxonomists of wood-rotting Basidiomycetes have been using cultural characteristics along with the macroscopic and microscopic characters as an aid to gain a further understanding of this diverse group of fungi.

Presented here for the first time are the cultural characteristics along with the key codes (Nobles, 1965) of three resupinate Basidiomycetes. The polyspore isolate of *Calocera cornea* (Fr.) Louden was obtained from Dr. A. E. Liberta who collected the original specimen in Williamson Co., Ill. The specimens of *Sebacina dubia* (Bourd. & Galz.) Bourd. and *Coniophora olivacea* (Fr. ex Pers.) Karst. from which polyspore isolates were made were collected by the author in Funks Grove, McLean Co., Ill. All specimen identifications were verified by Dr. A. E. Liberta.

The cultural methods and descriptive criteria used in this investigation are those of Nobles (1965). All cultures are deposited in the ISU culture collection.

Calocera cornea (Fr.) Loudon
Mat cream-white, turning orange in light, smooth in appearance, gelatinous in texture; hyphae simple-septate, thin-walled 6-8 X 3-4 u diam.; reacting negatively to both gallic acid and tannic acid; odor sweet and growth extremely slow.

Key Code: 1.6.7.33.36.(37).38.47.50.54.55.
Polyspore isolate. Culture No *ISU 70-46.*

Coniophora olivacea (Fr. ex Pers.) Karst.
Mat cream-like in color with margins fluffy and older areas appearing appressed in a reticulate fashion; hyphae with multiple clamp connections, thin-walled, with adherent brown resinous masses sometimes present; giving a negative extracellular oxidase reaction to gallic acid and a weak reaction on tannic acid.

Key Code: 1.5.(7).(21).35.36.(37).38.(39).43.(54).55.
Polyspore isolate. Culture No *ISU-57.*

Sebacina dubia (Bourd. & Galz.) Bourd.
Mat white, felty at first but becoming appressed with age; thin-walled hyphae with simple-septa found throughout; surface hyphae hyaline, 1.5-2 u diam.; tests for extra-cellular oxidase showing a strong positive reaction to both gallic acid and tannic acid.

Key Code: 2.6.7.32.38.46.47.54.55.
Polyspore isolate. Culture No *ISU-54* and *RMM 71-14.*

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Manuscript received August 21, 1971.

Notes

A NEW BISTETRACYCLONE. 2,2′-p-PHENYLENEBIS (3,4,5-TRIPHENYLCYCLOPENTADIENONE) AND ITS DIELS-ADLER PRODUCTS

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ABSTRACT.—A new bistetracyclone, 2, 2′-p-phenylenebis (3,4,5-triphenylcyclopentadienone) (3) was synthesized by the basic condensation of benzil and α,α′-bis (phenylacetyl)-p-xylene (2). Compound 3 reacted with phenylacetylene and dimethyl acetylenedicarboxylate to give the Diels-Alder products, 4 and 5, respectively.

In the course of studies aimed at producing a polytetracyclone polymer and some derived aromatic Diels-Alder polymers, it became necessary to prepare compounds 3, 4, and 5 for model compound studies.

The bistetracyclone (3) was prepared by the basic condensation of benzil with α,α′-bis (phenylacetyl)-p-xylene (2) in diethylene glycol solvent (Fig. 1). Compounds 4 and 5 were prepared by the Diels-Alder reaction of 3 with phenylacetylene and dimethyl acetylenedicarboxylate, respectively, in refluxing diglyme (Fig. 2). In the Diels-Alder reactions the products are formed by first giving the 1,4-cycloaddition products followed by the elimination of carbon monoxide to give the aromatic ring structure. These reaction sequences are well known and can be found in the literature in a detailed review by Ogliaruso and coworkers (Ogliaruso, *et al.* 1965). No attempt was made to separate and characterize the different isomers which are probably formed when phenylacetylene reacts with the bistetracyclone (3).

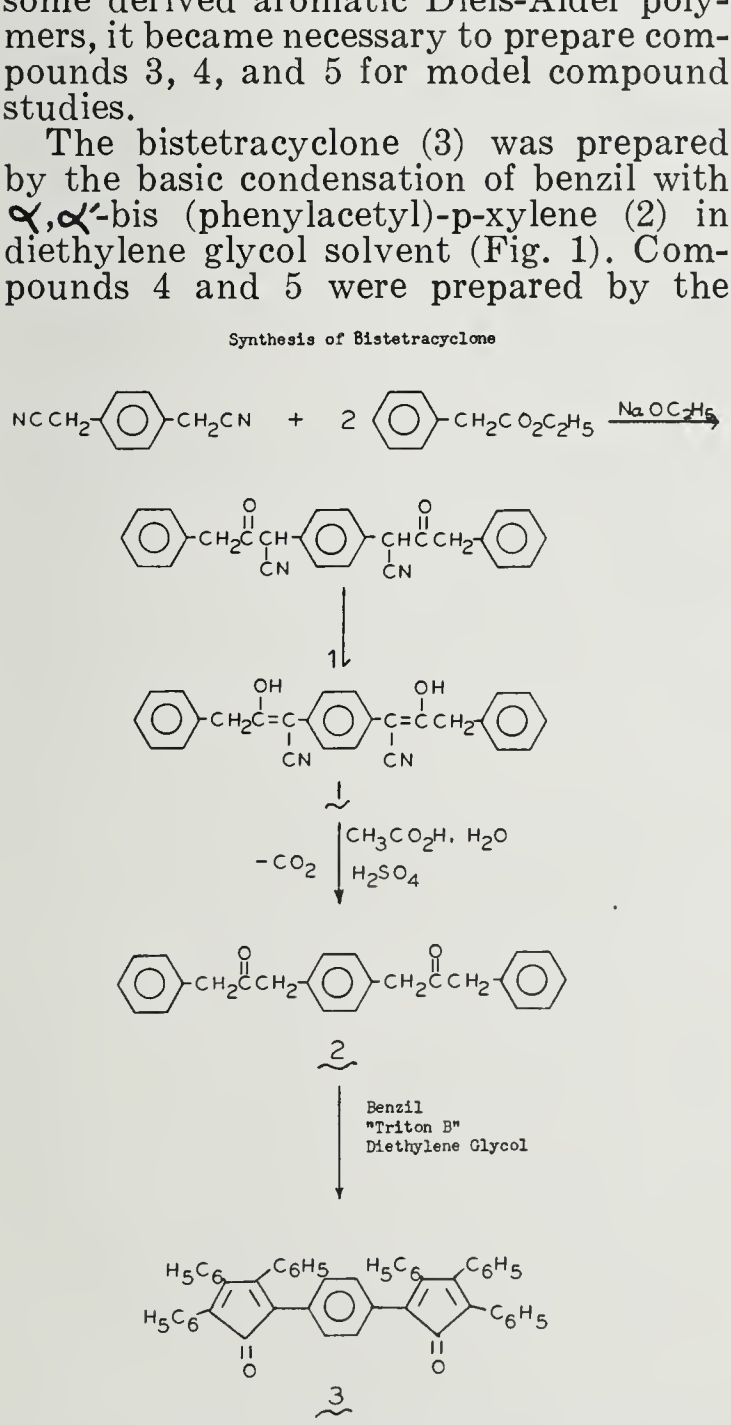

FIGURE 1.—Synthesis of Bistetracyclone.

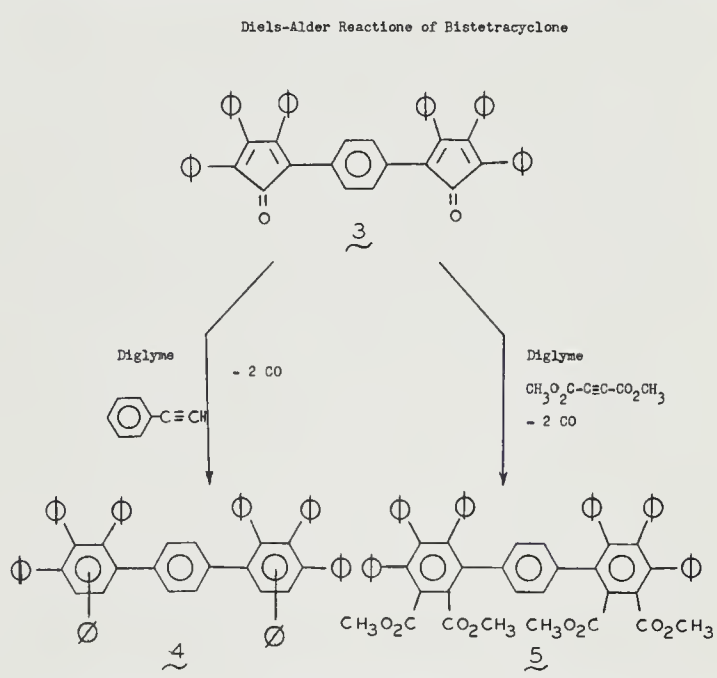

FIGURE 2.—Diels-Alder reactions of Bistetracyclone.

The basic condensation of p-phenylenediacetonitrile with ethyl phenylacetate produces the corresponding product (1) in approximately 60% yield. It is interesting to note that compound 1 exists almost completely in the enol structural form. This is evidenced by the absence of the carbonyl band and the presence of a strong O-H band in the ir spectrum.

EXPERIMENTAL

Preparation of Compound 1. - To a 1l round-bottom flask were added 25 g (0.16 mole) *p*-phenylenediacetonitrile, 164 g (1.0 mole) ethyl phenylacetate, 400 ml of

absolute ethanol, and 68 g (1.0 mole) sodium ethoxide. The solution was refluxed for 5 hr and then allowed to stand overnight. The ethanol solvent was removed with a vacuum evaporator and the residue poured into 1l of ice and stirred until all the ice melted (~ 1 hr). The solution was then extracted twice with 400 ml portions of ether to remove the excess ethyl phenylacetate. Acetic acid (125 ml) was then added to the aqueous solution and a thick oil separated which partially crystallized upon standing for about 0.5 hr. The crystals were removed by suction filtration and washed with methanol. The remaining oil was then dissolved in 500 ml of methanol and cooled at 0° for 48 hr, and a second crop of crystals was obtained to give a total of 37 g (59%) of product melting at 183-185°. A small portion was recrystallized from methanol for analysis (m.p. 186-187°). The remaining product was used without further purification. *Anal.* Calcd for $C_{26}H_{20}N_2O_2$: C, 79.55; H, 5.15; N, 7.13. Found: C, 79.40; H, 5.18; N, 7.00.

Preparation of Compound 2. - To 300 ml of glacial acetic acid was added 8 g (0.02 mole) of compound 1 and the solution refluxed until all of the compound dissolved. While the solution continued to reflux, 50 ml of 50% aqueous sulfuric acid was added slowly. The solution was then refluxed for 24 hr. The solution was cooled and added to 1l of cool water. The product was filtered and dried to give 6.2 g (90%) of crude product. The product was purified by recrystallization from 95% ethanol (m.p. 80-81°). *Anal.* Calcd. for $C_{24}H_{22}O_2$: C, 84.18; H, 6.47. Found: C, 83.99; H, 6.37.

Preparation of Compound 3. - To 50 ml of triethylene glycol were added 1 g of compound 2, 2 g of benzil, and 1 ml of 30% Triton B methanol solution. The temperature of the solution was raised to 130° and kept at this temperature for 30 min. The solution was cooled and filtered to give a quantitative yield of the *bis*-tetracyclone melting at 355-359°. *Anal.* Calcd. for $C_{52}H_{34}O_2$: C, 90.41; H, 5.03. Found: C, 90.29; H, 4.94.

Preparation of Compound 4. - To 50 ml of diglyme were added 0.5 g of compound 3 and 1 g of phenylacetylene. The solution was refluxed until the solution turned from a dark purple to a clear solution (30-45 min.). The solution was then cooled and poured into 200 ml of methanol. The methanol solution was cooled and the product filtered to give a quantitative yield of white product melting at 365-368°. *Anal. Calcd.* for $C_{66}H_{16}$: C, 94.47; H, 5.53. Found: C, 93.85; H, 5.42.

Preparation of Compound 5. - The exact same procedure was used as for the preparation of compound 4. The product melted at 338-339°. *Anal Calcd.* for C_{62} $H_{62}O_{16}$ C_8 81.03; H, 5.05. Found: C, 81.11; H, 4.89.

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Manuscript received August 21, 1971.

Notes

FIRST OCCURRENCE OF THE LEECH, *ACTINOBDELLA TRIANNULATA* IN ILLINOIS

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ABSTRACT.—The first occurrence of the leech, *Actinobdella triannulata* Moore, in Illinois is reported, along with a new host record in the United States. A full description of this leech is included. The literature on this fish parasite is vague and contains a number of discrepancies.

According to previous records, *Actinobdella triannulata* Moore, is a permanent fish parasite although on one occasion it has been reported as free-living. Moore (1924), who first described this species, reported that several were recovered from mud, sand and gravel dredgings taken from Lake Nipigon, Ontario, Canada. The presence of vertebrate blood in some of the specimens indicated they had been parasitic. *A. triannulata* is of special interest for it and *Placobdella pediculata* Hemingway are the only leeches of the Family Glossiphonidae (Rhynchobdellida) which are permanent parasites on fishes.

On June 18, 1971, eight specimens of this unusual and rare leech were collected from four hogsuckers, *Hypentelium nigricans* (LeSueur). The collection location was Owens Creek in the southwest quarter of Section 27, township 42 north, Range 3 East, DeKalb County, Illinois. Owens Creek flows north into the south branch of the Kishwaukee River, which flows into the Rock River, a tributary of the Mississippi.

The new specimens were attached to the inside of the opercula of the fish. Blood oozed profusely from the area of attachment when the leeches were removed. Closer inspection showed deep wounds, circular to bulbous cavities, caused by penetration and anchoring of the caudal sucker within the opercular tissue. The depth of these wounds, about 1.5 mm, indicated the leeches had probably parasitized the hogsuckers for a considerable time. A short distance from and around these holes were also smaller perforations which undoubtedly were sites where the leech had opened wounds with its proboscis and sucked blood and body juices.

Collections of fishes from the same location at a later date yielded three more specimens, from *H. nigricans*, and one from the common white sucker, *Catostomus commersoni* (Lacépède). All leeches were found on mature, good-sized fishes, the smallest being 25 cm standard length (about 10 inches).

A. triannulata has never been reported from Illinois, nor has it been recorded anywhere as a parasite of *H. nigricans*. Previous host records, though, show that it has a predilection for parasitizing fishes of the Family Catostomidae. At the present, records indicate that *A. triannulata* has been taken from: *Catostomus fecundus*, Wyoming, Bangham (1951); *C. catostomus*, *C. macrocheilus*, British Columbia, Bangham and Adams (1954); *C. commersoni*, Ontario, Meyer and Moore (1954); *C. catostomus*, *C. commersoni*, Lake Huron, Bangham (1955); *C. commersoni*, New Hampshire (U. S.) and Ontario, Meyer (1962).

DESCRIPTION OF *A. triannulata*

To the authors' knowledge this is the first time living specimens have been used in describing this species. Moore (1924) based his original description of this species on preserved specimens.

Size: small, normally extended living forms range from 4 mm to around 10 mm long (about three-eighths to less than one-half inch); unextended forms and most preserved specimens tend to be somewhat smaller due to abnormal contraction.

Shape and form (Fig. 1A): generally more ovoid and broadly blunt at anterior end, rather than flattened, roughly "spindly-shaped" or pear-shaped as is usual of glossiphonid leeches. The live specimens taken from the host were gorged with blood; they were convex dorsally, somewhat flattened ventrally. They tended to roll into a ball, a typical behavioral characteristic of several other glossiphonids.

Caudal sucker (Fig. 1B): in living forms bulbous and cup-shaped, considerably wider than the short, obvious sucker peduncle but narrower than posterior region of body; when preserved and sucker has been spread and flattened, it is large, nearly as wide as widest part of the body; inside lip of sucker possesses about 30 transparent papillae arranged concentri-

FIGURE 1.—*Actinobdella triannulata*. A, entire animal, B, caudal sucker.

cally; these papillae appear conical or finger-like, and are clearly evident in well-fixed specimens in which the sucker has been partially spread open and preserved in its characteristic bulbous shape; otherwise, they are often vague and difficult to see; one must use 20-30 magnifications and proper illumination to see them clearly.

Eyes: one pair, paramedial (as in several other glossiphonids), on segment III, dark pigmented and cupped.

Coloration: in living, adult forms, dark green to brown; in young specimens, lighter green to light brown and somewhat transparent; preserved specimens vary with killing and preserving techniques and length of time preserved; they range from light to dark greens or browns; dorsum with about 30 faint brownish, narrow, longitudinal lines, their clarity variable, usually evident but sometimes vague in living and recently preserved specimens, usually waning to obsolete in long-time preserved specimens.

Papillae (Fig. 1A): dorsum with conical, sharp-pointed papillae in five longitudinal rows, each papilla of the series being located segmentally; the median row the most obvious, the intermediate and submarginal rows less prominent; also arranged similarly are papillae which protrude marginally or dorso-marginally; the rows of papillae are clearly evident in living and well-fixed, naturally extended specimens, vague to indiscrete in poorly fixed and abnormally extended or flattened specimens.

Annulation: 3-annulate (as its name suggests); in the mid body region dorsally and ventrally the intersegmental furrows are very distinct, the intervening annular furrows less distinct and thus the annuli appear in distinct groups of three.

ACKNOWLEDGMENTS

The authors are indebted to Carol Lovekin for her drawings and to Robert Langellier and Thomas Hiebert for their assistance in collecting the fish.

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Manuscript received December 2, 1971.

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PREPARATION OF MANUSCRIPTS FOR THE TRANSACTIONS

For publication in the *Transactions*, articles must present significant material that has not been published elsewhere. Review articles are excepted from this provision, as are brief quotations necessary to consider new material or varying concepts. All manuscripts must be typewritten, double spaced, with at least one-inch margins. The original copy and one carbon copy should be submitted.

Titles should be brief and informative. The address or institutional connection of the author appears just below the author's name. An abstract must accompany each article. Subtitles or center headings should be used; ordinarily one uses subtitles such as *Materials and Methods*, *Results*, *Discussion*, *Summary*, *Acknowledgments*, and *Literature Cited*.

No footnotes are to be used except in tables.

The section entitled *Literature Cited* must include all references mentioned in text. It is not to include any other titles. Citations under *Literature Cited* are as shown below:

Doe, J. H. 1951. The life cycle of a land snail. Conchol., 26(3): 21-32, 2 tables, 3 figs.

Doe, J. H., and S. H. Jones. 1951. Mineralogy of Lower Tertiary deposits. McGraw-Hill Book Co., New York. iv + 396 pp.

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TRANSACTIONS

OF THE

ILLINOIS STATE ACADEMY OF SCIENCE

VOLUME 65 - 1972 Nos. 3 and 4

ILLINOIS STATE ACADEMY OF SCIENCE
AFFILIATED WITH THE
ILLINOIS STATE MUSEUM DIVISION
SPRINGFIELD, ILLINOIS

(PRINTED BY AUTHORITY OF THE STATE OF ILLINOIS)

Sept. - Dec., 1972

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THE MECHANISM OF CARBOHYDRATE TRANSLOCATION IN STRAIGHT-NECK SQUASH

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ABSTRACT.—An investigation concerning the localization of stachyose in petioles of straight-neck squash (*Cucurbita pepo* L. var. *melopepo torticollis* Bailey) was conducted. Chromatographed extracts of two-centimeter petiole sections from nodes two and three of 22-day squash were analyzed colormetrically for the concentration of stachyose and sucrose, both major transport molecules in this plant. The concentration of stachyose per petiole, assuming that all the stachyose was in the phloem and evenly distributed therein, was calculated to be between 0.3 and 0.4 molar. The molar concentration of sucrose in the phloem was calculated to be between 0.25 and 0.35. Isolation of the majority of the stachyose in a particulate cell fraction prepared from petioles was accomplished by means of a fructose density-gradient. These results are consistent with the hypothesis that phloem transport occurs by means of particulates which move in or on transcellular strands.

The major hypotheses concerning the mechanism of carbohydrate translocation in higher plants may be listed as follows: (1) mass flow, (2) electroosmosis and mass flow, and (3) protoplasmic streaming. The latter hypothesis has been extended by Thaine (1969) to include the transcellular streaming concept which assumes that the transported molecules move through endoplasmic tubules within the transcellular strands. These strands, 1 to 7 u in diameter, have been observed in many plants, including straight-neck squash (Thaine, 1969), although Esau *et. al.* (1963) interpret these strands as "merely lines caused by diffraction of light from walls out of focus." Such a highly structured tubular system would be expected to be highly susceptible to rapid environmental changes such as cooling. In a recent study concerning chilling and translocation, Geiger (1969) concluded that metabolic dependence in the path is limited to the need for energy to maintain the structural integrity of the translocation channels.

Stachyose is a major transport molecule in squash (Pristupa, 1959; Webb and Gorham, 1963); data reported by Weidner (1964) may be interpreted to indicate that the majority of the stachyose in squash is localized in the phloem. This paper reports supplementary data which also suggest localization of stachyose in the phloem. In addition, results are presented which indicate further localization of this sugar on cell particulates which may be associated with the transcellular strands.

MATERIALS AND METHODS

Seeds of *C. pepo* L. var. *melopepo torticollis* Bailey were germinated in a moist chamber for four days and were then maintained on cheesecloth over an aerated 10^{-4}M $CaSO_4$ solution for an additional four days. Seedlings were transferred to an aerated mineral solution in a controlled environment chamber. The photoperiod was 16 hours light (24° ± 1 C.) and 8 hours dark (19° ± 1 C.). Light intensity from fluorescent and incandescent lamps was 1,000 foot-candles measured six inches above the top of the culture tanks.

On the day of each experiment (22 days from the time the seeds were placed in the moist chamber) the petioles from nodes two and three (counting from the bottom of the plant) were removed and sectioned into two-centimeter lengths. The first centimeter of petiole closest to the

stem was not used due to the reported accumulation of sugars in this region (Fisher, 1970). After weighing, each section was extracted separately in a soxhlet extractor with 80% (v/v) ethanol in water for three hours. Extracts were stored in a freezer at -25° C.

Sugars in the extract were separated by means of descending paper (Whatman #1) chromatography in a 1-butanol: acetic acid: water (3:3:2) solvent system for 24 hours. Location of individual sugars was accomplished by co-chromatography with known sugars on side strips on the chromatograms. Sugars on side strips were visualized by means of a benzidine spray according to the method of Bacon (1951). Strips containing the individual sugars were cut from the chromatograms and eluted with water into a test tube. Quantitative determinations were accomplished with an anthrone reagent (100 mg of anthrone in 50 ml of concentrated sulfuric acid). Three ml of the reagent were added to one ml of aqueous sugar solution. The mixture was incubated at 100° C. for ten minutes, then cooled to 25° C. and read in a Spectronic 20 colorimeter at 620 nm. A standard curve was prepared for each of the sugars. Total sugars reported in this paper are an approximation since the relative concentration of the several sugars in each extract varied, although stachyose and sucrose accounted for the majority recovered from petioles in all cases.

Fructose density-gradients were used in attempts to isolate cell particulates rich in stachyose because the fructose separated from stachyose in the chromatography system described above. Linear gradients (100 mg to 350 mg per ml) were prepared in 15-ml plastic centrifuge tubes and stored at 5° C. for one hour prior to use. Cell particulates were obtained by grinding petiole sections in a Ficoll-mannitol buffer (0.25 M mannitol, 2.5 g. Ficoll/100 ml of solution, 5mM $MgCl_2$, 0.025 M Tris-HCL, pH 7.0) which was modified from that reported by Honda (1966). The homogenate was filtered through four layers of cheesecloth and centrifuged at 1000 x g for five minutes. The supernatant (1.5 ml) was layered onto the cold gradient which was then centrifuged for three hours at 25,000 x g in a Sorvall HB-4 swinging cup rotor. One-ml fractions of the gradients were collected and 0.1 ml aliquots of each were analyzed for stachyose by decending paper chromatography and the anthrone reagent as outlined above. Streaking of fructose on the chromatograms prevented accurate sucrose determinations on these same gradient aliquots.

Phloem volume was determined from free-hand cross-sections of the petioles stained with 1% aniline blue; the area of the internal and external phloem was measured along the length of the petioles.

Results and Discussion

The results of the sugar determinations are shown in Table 1. Both stachyose and sucrose concentrations (per petiole) decrease from the base of the petiole toward the leaf blade. Both tissue and phloem volumes also decrease from petiole base to blade. Sucrose concentration is usually 30 to 50 percent that of stachyose in the petiole. These two transport sugars account for the majority of the sugar in the petiole, as would be expected in a structure whose basic function is translocation.

The decreased proportion of phloem in the leaf blade compared to that in the petiole is reflected in the fact that total sugar per gram fresh weight increases in the leaf blade while the relative concentration of the transport sugars, especially stachyose, decreases. This indicates that stachyose

TABLE 1.—Comparison of total sugars and transported sugars (stachyose and sucrose) in 2-cm sections of petioles from nodes 2 and 3 and in blades from 22-day straightneck squash.

node	cm section	weight (g)	total sugars* (mg)	stachyose (mg)	sucrose (mg)
2	2	0.52	1.2	0.84	0.29
	4	0.43	0.9	0.63	0.21
	6	0.36	1.2	0.77	0.25
	8	0.31	1.0	0.77	0.25
	10	0.27	1.0	0.7	0.25
	12	0.23	0.6	0.45	0.17
	14	0.19	0.53	0.35	0.15
	16	0.17	0.53	0.35	0.17
leaf blade	—	1.00	7.0	0.88	1.58
3	2	0.58	1.23	0.84	0.21
	4	0.50	1.0	0.7	0.25
	6	0.42	1.5	0.98	0.33
	8	0.38	1.23	0.63	0.37
	10	0.33	1.13	0.77	0.37
	12	0.30	0.73	0.63	0.20
	14	0.26	0.67	0.56	0.33
	15.58	0.18	0.67	0.56	0.17
leaf blade	—	1.00	8.0	0.98	1.74

*approximate (see text)

is more restricted in its distribution than is sucrose, the former sugar possibly being restricted to the transport system in squash.

Measurements indicated that average volume of internal and external phloem per petiole to be 20 mm^3. A comparison of the amount of stachyose per petiole (4.9 mg) with the volume of the phloem indicated that the molarity of stachyose was between 0.3 and 0.4 if all the stachyose were contained in the phloem. These data appear consistent with those of Zimmerman (1957) from analysis of white ash sap exudate where he determined the molar concentration of stachyose to vary between 0.15 and 0.3, depending on diurnal and seasonal fluctuations. The stachyose concentration in squash calculated above was greater than that reported by Zimmerman, but white ash has relatively high concentrations of mannitol (0.05 to 0.2 M) in the phloem along with stachyose. The calculated molar concentration of sucrose in squash was determined to fluctuate between 0.25 and 0.35. This is higher than Zimmerman's figures but again may be explained if one considers the additional carbohydrates in white ash. Thus, the total molar concentration of carbohydrates calculated by Zimmerman was similar to that reported above.

If the stachyose were being transported in or on organelles which were moving through the transcellular strands. then one should be able to isolate stachyose-rich particulates from a petiole cell homogenate. Figure 1 shows results of density-gradient separations of particulates prepared from homogenates of selected squash petiole sections. The 2 to 5 ml fraction of the gradient contained 50 percent of all the stachyose in the petiole section. The unlayered sediment contained 20 to 30 percent of the stachyose in the petiole section and the remainder was in the other gradient fractions.

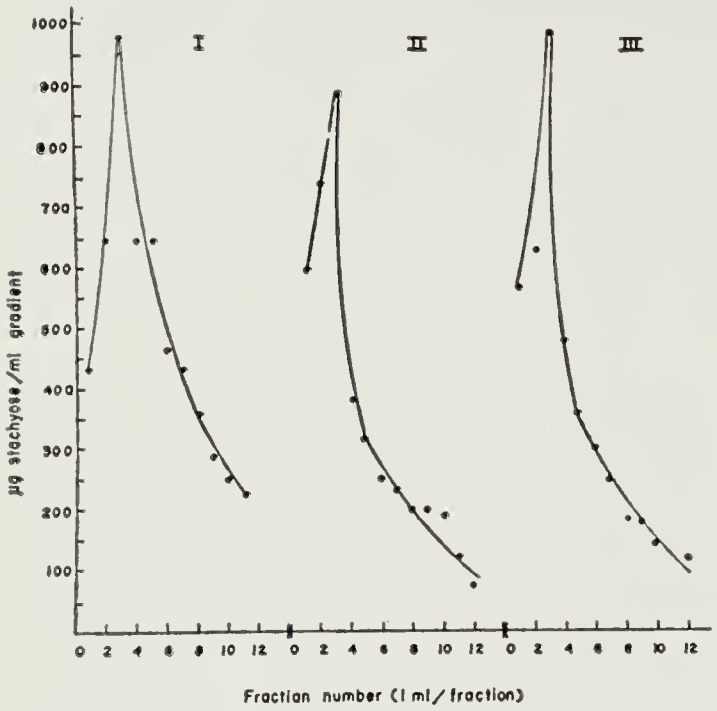

FIGURE 1. Stachyose concentration in the several fractions of the density-gradients.

The mechanism of translocation proposed by Canny (1962) and later modified by Thaine (1969) suggests that transport in sieve tubes occurs within strands of cytoplasm where liquid may flow bidirectionally. The space between the strands is, according to theory, a carbohydrate reservior which is in rapid equilibrium with material inside the strands. An extension of this hypothesis suggests that the translocated carbohydrates are carried in or on organelles which flow through these transcellular strands. Thus, in squash petioles, where most of the stachyose is apparently in transit, one would expect to find most of the stachyose associated with cell particulates. The data presented in this paper appears to support this elaboration of the transcellular streaming hypothesis of carbohydrate translocation.

ACKNOWLEDGMENTS

This research was supported in part by a grant from the Council on Faculty Research at Eastern Illinois University.

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Manuscript received May 18, 1971.

INFLUENCE OF *CLOSTRIDIUM BOTULINUM* TYPE A SPORES AND TOXIN ON CIRCULATING BLOOD LEUKOCYTE VALUES OF SWISS MICE.

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ABSTRACT.—Mouse peripheral leukocyte values were elevated following intravenous challenge of *Clostridium botulinum* spores (10^6/mouse) and botulinal type A toxin (1 LD_{50}/mouse). An initial decrease in leukocyte values was observed with the bacterial spores, but not with toxin. However, after 10 min., a leukocytosis was present in both cases: 15,000 cells/mm^3 at 18 hr. for *C. botulinum* type A spores and 12,500 cells/mm^3 at 18 hr. for botulinal type A toxin.

It is possible that the leukocytosis induced by spores might render the host more susceptible to "botulism" pathogenicity through increased induction of germination and toxin release of *C. botulinum* spores by leukocytes.

Previous investigations (Grecz and Lin. 1967; Booth, *et al,* 1971a) have demonstrated that *Clostridium botulium* type A spores, when injected intraperitoneally into mice, cause death of the animal from botulism. Therefore, it can be surmised that spore-bound toxin is released *in vivo.* The mechanism of this toxin release has been elucidated recently: (i) *In vitro* guinea pig leukocyte- *C. botulinum* spore suspensions convert the heat-resistant (80 C, 15 min) spores to heat-sensitive germinated spores concomitantly releasing botulinal toxin at 8 hr (Suzuki, *et al,* 1970); (ii) leukocyte-induced germination of *C. botulinum* spores can be observed by staining of peritoneal exudates (Booth, *et al,* 1971b); and (iii) using release of ^{45}Ca from labeled spores as an indicator of germination, the radioisotope was detected in the free state beginning at 8 hr when guinea pig leukocytes were incubated with labeled spores (Suzuki, *et al,* 1971a). These findings relate *C. botulinum* spore pathogenicity to phagocytic engulfment by leukocytes, subsequent spore germination and phagocytic digestion resulting in release of spore-bound toxin.

Since leukocytes play a major role in this type of botulism pathogenicity, peripheral blood leukocyte values become important in understanding the course of the disease in addition to offering some plausible explanation for fatalities resulting from wound infections caused by *C. botulinum* (Dolman, 1961; Thomas *et al,* 1951; and Davis *et al,* 1951). It has been variously proposed that botulinal toxin appears in the bloodstream due to interaction of *C. botulinum* spores with leukocytes (Suzuki, *et al,* 1970; 1971a), due to gastrointestinal growth of the organism as asserted by Soviet workers (Minervin, 1962; Shvedov, 1959; and Shvedov, 1960), or due to cell autolysis in the gastrointestinal tract and subsequent release of toxin (Lamanna and Carr, 1967). In any case, it is important to ascertain the effect of this toxin on the mobilization of leukocytes into the circulating bloodstream since a leukocytosis may enhance pathogenicity of these Clostridia spores.

MATERIALS AND METHODS

Culture Methods

A culture of *Clostridium botulinum* type A strain 33 A was inoculated aseptically into an incubation medium of 5% Trypticase (Baltimore Biological Laboratories, Baltimore, Md.),

1% peptone (Difco, Detroit, Mich.) and 0.1% sodium thioglycollate. The methods for harvesting and eliminating vegetative cell contaminants have been described (Grecz, *et al,* 1961). Harvested spores were stored in 0.85% NaCl at 4 C until needed.

Experimental Animals

Swiss-Webster strain mice of 20-25 grams were used in the experiment. All mice were inbred for 12 generations in a thermostatically controlled animal room at the Illinois Institute of Technology and were watered and fed *ad libitum* with Rockland Rat and Mouse Diet.

Blood Samples and Injections

The mice were undisturbed for a minimum of 72 hr prior to intravenous injections. This was necessary because it has been reported that stress effects leukocyte in this animal (Jensen, 1969). Therefore, it was ascertained that mice were not aggravated by any kind of stress for a minimum of 72 hr prior to experimentation. Mice were used only once for blood sampling. Since sex (Halberg, *et al,* 1957) and age (Russel, *et al,* 1951) of mice affects blood studies, all mice used in the expermient were of male sex as recommended by Chapman (1968) and were a minimum of 9 weeks old as recommended by Rugh and Somogyi (1968). For studies of leukocyte values, intravenous (IV) injections were made into the dorsal tail vein of the mouse with a 1 ml disposable glass syringe equipped with a 26 gauge needle. All blood samplings were taken from the tail at 3:00 P.M., a time when leukocytes in the tail vein blood of mice were found to be most prevalent (Brown and Dougherty, 1956).

Blood Cell Counts and Differentials

Free-flowing tail vein blood was drawn up in an Unopette white blood cell diluting pipette (Becton-Dickinson, Rutherford, N.J.) and counted in the same hemocytometer by standard clinical laboratory procedures. A smear of the blood from the same tail vein was Wright's stained and a differential of leukocytes (expressed as %) calculated from 200 cells observed.

Botulinal Type A Toxin

Crystalline toxin ($S_{20,w}$7.1) was obtained courtesy of Dr. E. J. Schantz, Department of the Army, Fort Detrick, Frederick, Md., and diluted with Krebs-Ringer Phosphate Medium, pH 7.4 (KRPM) to a final concentration of 1 MLD_{50}/ml. At this concentration, approximately 50% of IV challenged mice, expire from botulism within 12-18 hr.

TABLE 1.—Leukocyte Values[a] for Normal and Control Injected Mice.

Injected	Leukocyte count (per MM^3)	PMN[b]	lympho-cyte	eosino-phil	mono cytes	bands[d]
none	7,610 ± 265	22 ± 3	69 ± 4	3 ± 1	2 ± 1	4 ± 1
KRPM[c]	8,532 ± 317	24 ± 3	67 ± 7	3 ± 1	2 ± 1	4 ± 1
BSA (0.023 μg/ml)[e]	8,981 ± 331	28 ± 4	66 ± 5	1 ± 1	3 ± 1	5 ± 2
Denatured toxin[f]	9,322 ± 359	27 ± 3	63 ± 5	2 ± 1	2 ± 1	6 ± 2

[a] 12 hr interval between injection and blood sampling, average of 15 animals with SEM.
[b] PMN = polymorphonuclear leucocyte. No significant numbers of basophils seen.
[c] Krebs-Ringer Phosphate Medium, pH 7.4.
[d] Bands were counted as any immature form of granulocytes.
[e] Bovine serum adbumin.
[f] Autoclaved botulinal toxin — 121 C for 20 minutes.

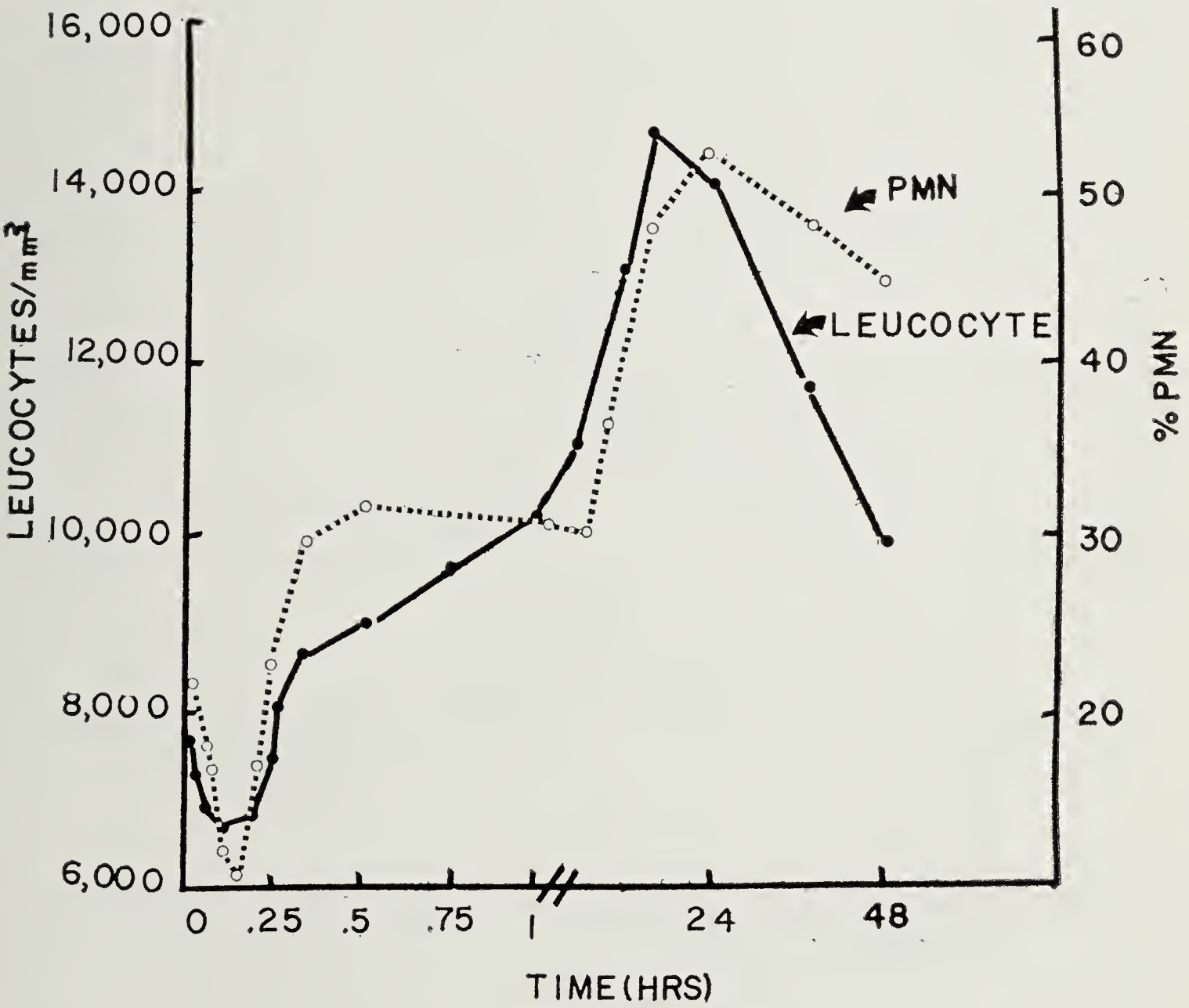
Bovine Serum Albumin (BSA)

Purchased from General Biochemicals, Chagrin Falls, Ohio, and diluted to 0.023 μg/ml with KRPM.

Results and Discussion

Normal values of leukocyte count and differential of the Swiss-Webster strain mice are shown in Table 1. These findings are within range of previous reports on mice (Dittmer, 1961; Russel and Bernstein, 1966). Control IV injections of KRPM, BSA (0.023 μg/ml), and denatured 1 LD_{50}/ml botulinal toxin (autoclaved at 121 C for 20 min) altered leukocyte counts and differential from non-injected mice. Denaturation of botulinal toxin was confirmed by mice toxicity tests.

A time course evaluation of leukocyte values and differentials in response to IV injected *C. botulinum* spores (10^6) is illustrated in Figure 1. *C. botulinum* spores depress leukocyte values at 5-10 min; this decrease is

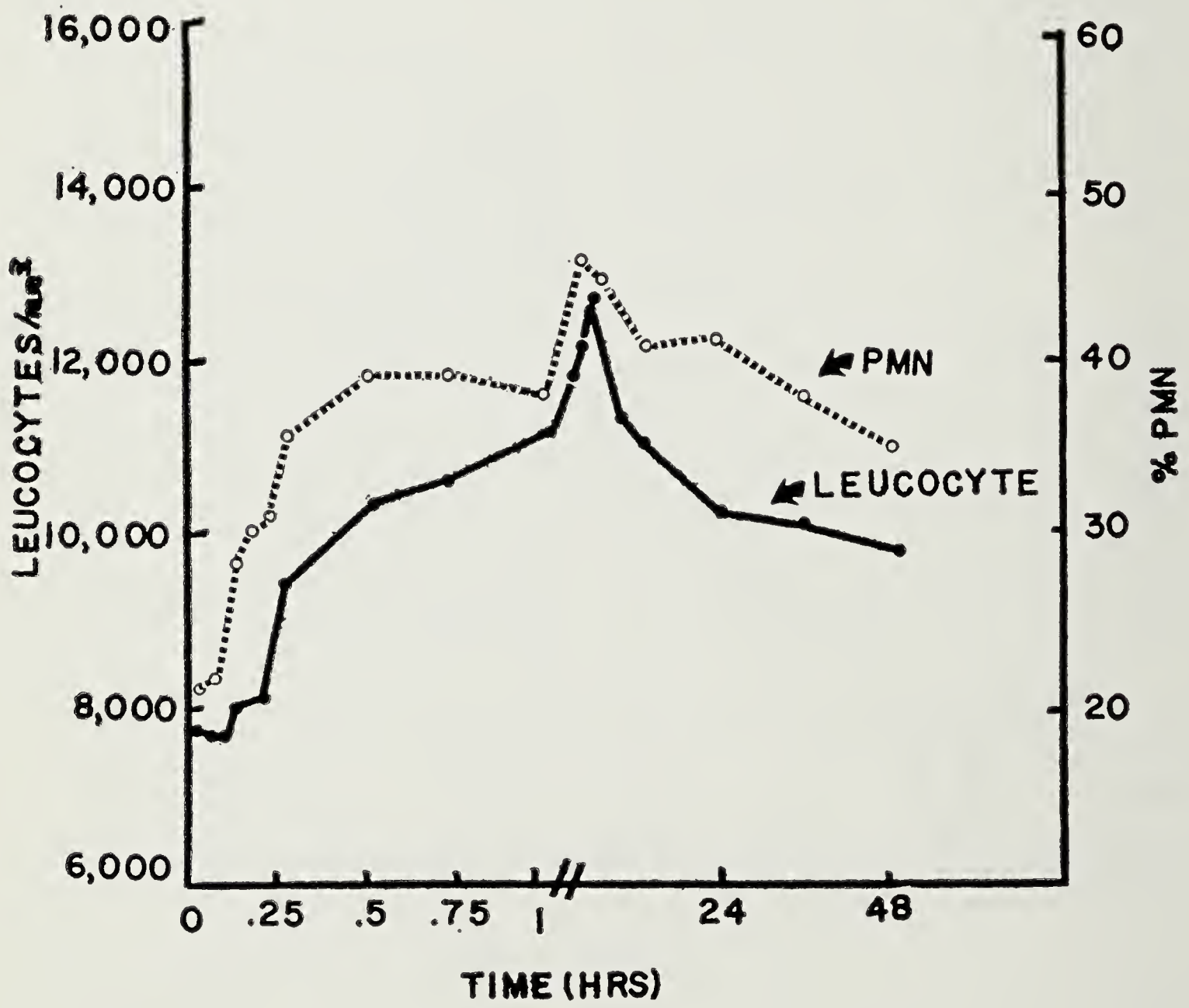


[a]average of 15 mice per time point(less than 12 hrs)
average of 5 mice(12 hrs and greater). (p=0.06).

Figure 1.—Leukocyte and PMN Values of Mice After IV Injection of 10^6 *C. botulinum* Spores[a].

followed by an increase to 14,700 cells/mm^3 by 12-24 hr. Leukocyte values approach normal counts at 48 hr. The decrease in leukocyte values, as well as percentage of PMN's in the differential at 1-10 min with IV injected *C. botulinum* spores, is in agreement with Rogers (1960) who reviewed leukopenia in rabbits after injections of either staphylococci or pneumococci. Of interest in the leukocyte differential are (i) an initial decrease in PMN leukocytes between 1-15 min; (ii) an increase in PMN leukocytes beyond normal values at 20 min and countinuing up through 24 hr; and (iii) an increase in immature cells (bands) also at 20 min but subsiding at 24 hr. Mice fatality rate for 10^6 IV injected spores was 37% at 48 hr.

The effect of botulinal toxin on peripheral leukocyte values is significant since the toxin has been repeatedly demonstrated in the peripheral bloodstream of botulinic victims (Hil-

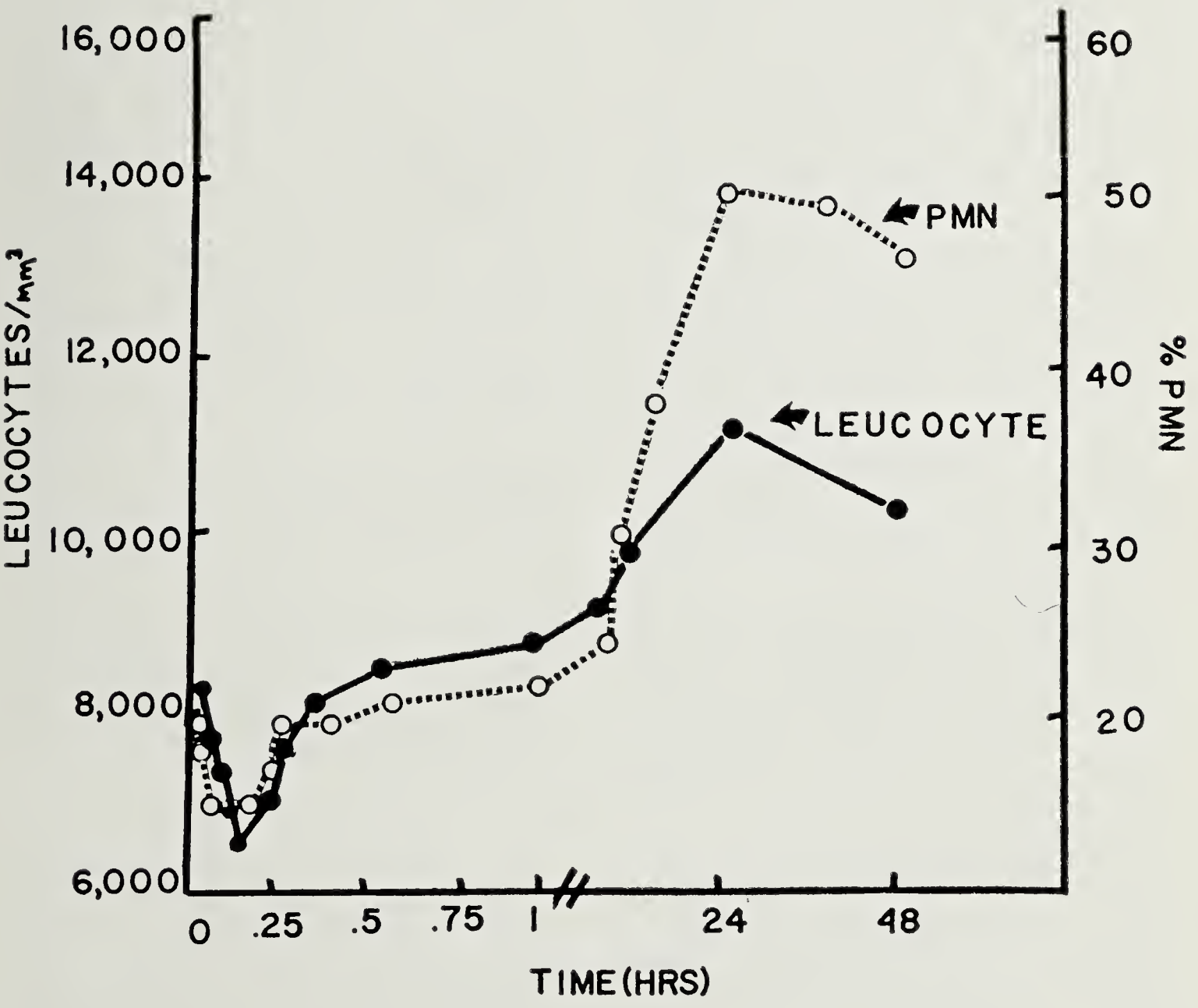


[a]average of 15 mice per time point(less than 12 hrs)
average of 5 mice(12 hrs and greater)., (p=0.06).

FIGURE 2.—Leukocyte and PMN Values of Mice After IV Injection of 1 LD_{50}/ml Botulinal Toxin[a].

debrand, *et al,* 1961; May and Whaler, 1958; and Dickson, 1926). As shown in Figure 2, injection of 1 LD_{50} of botulinal type A toxin causes no depression of leukocyte value or PMN of the differential during the initial 5-10 min. However, leukocyte values increased dramatically to 12,800 cells/mm^3 at 4 hr, similar to those seen with spores. Again an increase in immature cells (bands) at 15 min was observed. Injection of 1 LD_{50} of botulinal toxin resulted in a 65% mice fatality at 48 hr. A leukocytosis has also been observed in response to anthrax toxin (Fish, *et al* 1968) and *Bacillus cereus* (Burdon, *et al,* 1967).

IV injections of 10^6 heat killed (100 C, 120 min) *C. botulinum* spores were included (Figure 3) to test the influence of the particulate spore matter as opposed to viable spores and 1 LD_{50} toxin. Death of spores was confirmed by subculture techniques (Wynne, *et al,* 1955). Within the first hr, mouse responses were iden-



[a]average of 15 mice per time point(less than 12 hrs)
average of 5 mice(12 hrs and greater).

Figure 3.—Leukocyte and PMN Values of Mice After IV Injection of 10^6 Heat-killed *C. botulinum* Spores[a].

tical whether injected with heat-killed spores or with viable spores, i.e., leukocyte values were depressed between 2-10 min. subsequent to which there was a gradual leukocytosis. However, leukocyte and PMN values induced by heat-killed spores never attained levels tantamount to those elicited by viable spores or toxin.

Conclusions

It is interesting to note that mouse peripheral leukocyte values respond differently to bacterial cells (*C. botulinum* spores) than to a bacterial toxin (botulinal). The initial decrease in leukocyte values upon administration of spores is probably due to a response of the natural defense mechanism of the host, viz. leukocytes adhering to the endothelium of blood vessels permitting more effective clearance of foreign particles. An increase in immature cells (bands) at 20 min through 24 hr implicates an activation of hemopoietic tissues (e.g. bone marrow) resulting in the influx of more phagocytic cells (though immature) into the peripheral bloodstream. It is documented that immature cells as young as myeloblasts, and promyelocytes have the ability to engulf particulate matter (Handler, *et al,* 1969; Ulbrich and Wiedemann, 1954; and Wang-Peng, *et al,* 1967). This is significant since engulfment of *C. botulinum* spores was demonstrated to be a requisite for botulinic pathogenicity.

Although similar increases in leukocyte count and shift to more immature forms are observed with IV administration of botulinal toxin, they are not as severe as with *C. botulinum* spores suggesting a mechanism by which the host differentiates between particulate matter and soluble foreign proteins.

It is plausible to apply these findings to cases of wound infection by *C. botulinum.* Ruptures in the skin of a host elicit an inflammatory response involving a complicated sequence of events which includes PMN leukocyte migration to the wound site. If PMN leukocytes at the wound site engulf *C. botulinum* spores and induce them to germinate and to release toxin as demonstrated *in vitro* (Suzuki, *et al,* 1970, 1971b), then increased peripheral leukocyte values could conceivably make available more leukocytes at an infected wound. We can speculate then, that increased numbers of leukocytes at the wound site could participate in greater induction of *C. botulinum* spore germination and toxin release, and that this could lead to fatal consequences if sufficient numbers of spores were found in the wound. However, should anaerobic necrotic conditions in the wound cause the spores to germinate and to grow into vegetative cells, then *C. botulinum* autolysis (Lamanna and Carr, 1967) or immediate destruction by phagocytes (Suzuki, *et al,* 1970) could release botulinal toxin. Continual parasitic growth of the organism or increased contamination of the wound by *C. botulinum* could then result in fatality of the host. It is suggested that the leukocytosis induced by spores and/or botulinal toxin renders the host more susceptible to "botulism" pathogenicity through increased induction of germination and toxin release of *C. botulinum* spores by leukocytes.

Acknowledgments

The excellent technical assistance of Andrew Benedik, Chicago Osteopathic School of Medicine, Chicago, Illinois is greatly appreciated. This research was supported by Public Health Service grant FD-00358, from the Food and Drug Administration, Consumer Protection and Environmental Health Service.

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Manuscript received September 22, 1971.

SEASONAL ORGAN WEIGHTS OF THE GREEN FROG, *RANA CLAMITANS* (ANURA, RANIDAE), UNDER NATURAL CONDITIONS

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ABSTRACT.—This study utilized 474 green frogs which were collected in weekly samples throughout an entire year in southern Illinois. The annual organ weight fluctuations were closely correlated with observed annual activities. Mean monthly liver weights reached two peaks during the year, one preceding the breeding season in June and the second just before hibernation in November. Low mean monthly values occurred in August, which terminated the reproductive period, and in February, the last month of winter. Statistically significant liver weight differences appeared between age groups and between adult sexes during the three month breeding period. Fat body weights, though extremely variable within any one month, tended to coincide with the annual liver weight oscillations. Oviduct and gonadal hypertrophy coincided with sexual activity. June and July marked the maximum reproductive readiness of females, while testes were heaviest during the period of May through August.

Although the organ weights of several anuran species have been studied (Bush, 1963; Houssay, 1949; March, 1937; Mizell, 1965; Smith, 1950), little data are available for annual organ weight fluctuations of North American frogs having extended breeding seasons. The present study provides the first 12 month organ weight analysis for the green frog, *Rana clamitans,* which has a three month breeding period. The organ weight data were gathered from a single population under field conditions. The resulting seasonal fluctuations in green frog organ weights reflect the physiological demands placed upon the species by naturally occurring phenomena. At times the seasonal organ weight trends of the green frogs deviated remarkably from those of other studied anuran species having different life histories. Though primarily descriptive, this study points out areas where causal agents may be investigated by future studies.

METHODS AND MATERIALS

A total of 474 frogs were taken from a single population located in and about LaRue Swamp in the Pine Hills region, Union County, Illinois. The frogs were collected in weekly samples from March, 1963 through February, 1964. The collection site was a permanently inundated swamp originating from an old oxbow of the Mississippi River and now fed by the watershed and springs. The vegetational characteristics of the area have already been described (Ashby and Kelting, 1963; Molhenbrock, 1959).

The frogs were brought back to the laboratory alive within three hours after capture; there they were placed in plastic bags and frozen. During processing only a few animals were thawed at a time. They were kept in wet paper toweling to prevent desiccation. Total body weight was determined to the nearest 1/10 g on a Harvard Trip Balance.

Weights were obtained for the liver, fat bodies, each oviduct and each gonad. As an organ was removed, it was blotted of extraneous fluid and weighed to the nearest 10 mg on a Roller Smith Precision Balance. When determining weights of particularly heavy organs such as ripe ovaries, counter weights were used. As the annual sample contained ovaries bearing eggs of all stages of development and since at no time were eggs found

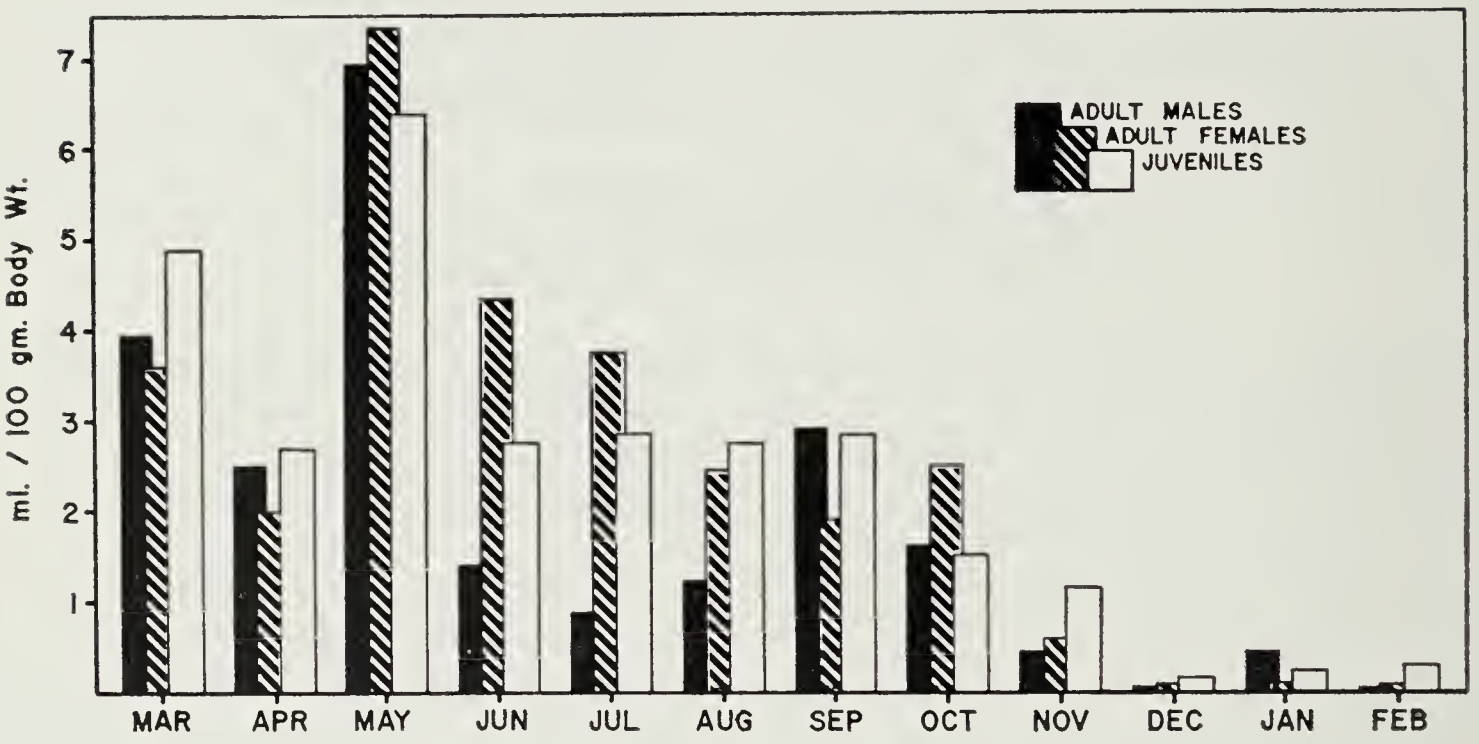

FIGURE 1.—Mean monthly volumes of food (expressed as ml/100 g eviscerated body weight) in the stomachs of 474 green frogs collected March 1963 to February 1964 in southern Illinois.

completely free in the coelom, all ovary weights include eggs.

Three categories were established for comparative purposes: juvenile, adult male, and adult female. Classification of the frogs into these three groups was based on a combination of the following factors: snout-vent length, appearance of secondary sexual characteristics, time of year, and an examination of the gonads. Male and female juvenile frogs were combined into one class as "t" tests showed no statistical differences between their corresponding organ weights (gonads excluded).

In order to compare different sizes of frogs, organ weights were divided by the eviscerated body weight of the frog from which they were taken. This also eliminated any weight bias resulting from adult gonads during the breeding season.

Volumes of ingested food (Fig. 1) were recalculated from data used by Jenssen and Klimstra (1966). Annual air temperature readings (Fig. 2) were recorded by the U.S. Weather Bureau in Cobden, Illinois which is located approximately 10 miles east of the study area.

RESULTS AND DISCUSSION

The annual behavior of the green frog is closely correlated with its seasonal organ weight fluctuations. The frogs' general activity can be divided into four 3-month periods. Beginning in March, the frogs emerged from hibernation and from this time through May underwent a period of intense feeding (Fig. 1). Maximum breeding activity occurred from June through August. Then in the fall, with decreasing air temperatures (Fig. 2), a build-up of energy reserves took place in the form of enlarged livers and fat bodies. From December through February most of the frogs hibernated. Seasonal behavior as well as morphological characteristics of the complete sample are given in greater detail elsewhere (Jenssen, 1968).

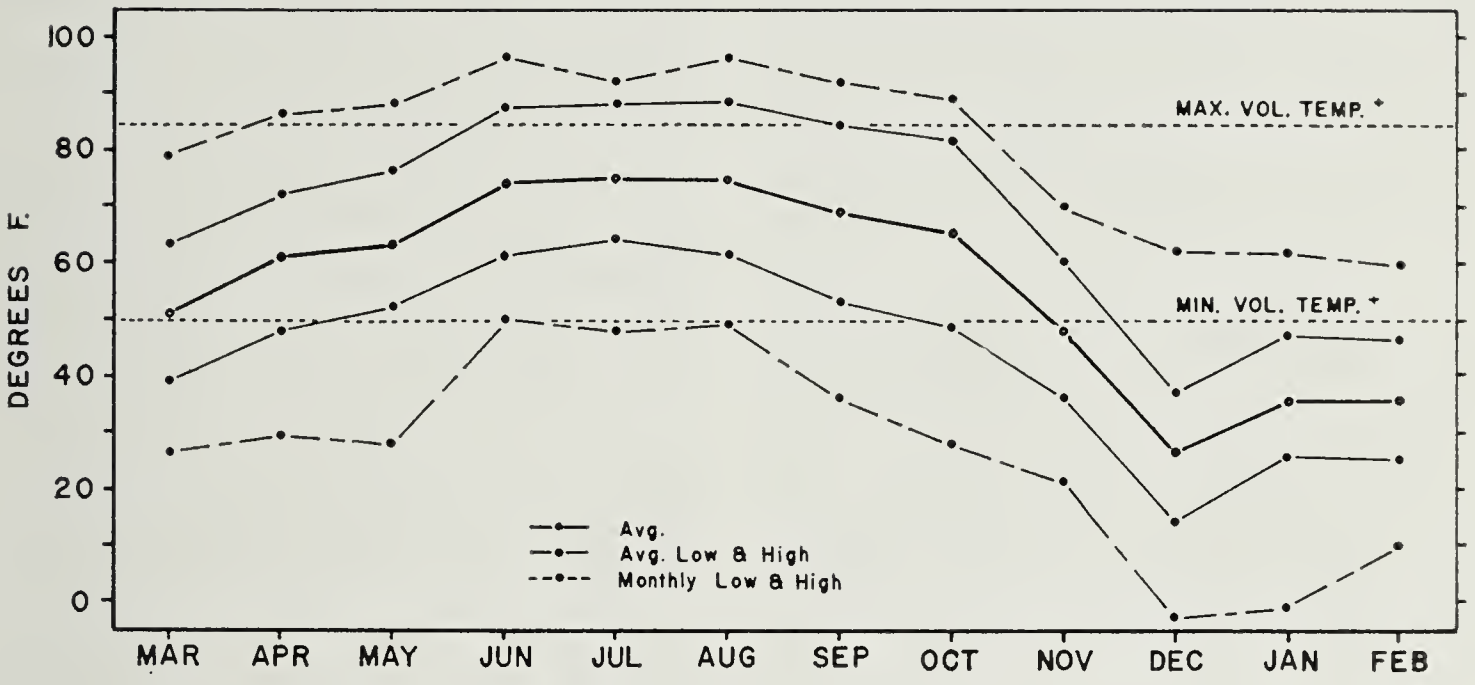

FIGURE 2.—Average, average low and high, lowest and highest air temperatures per month during March 1963 to February 1964 in Cobden, Union Co., Illinois, approximately 10 miles east of study area.

*Max.-min. voluntary temperature limits for *R. clamitans*, (Brattstrom, 1963)

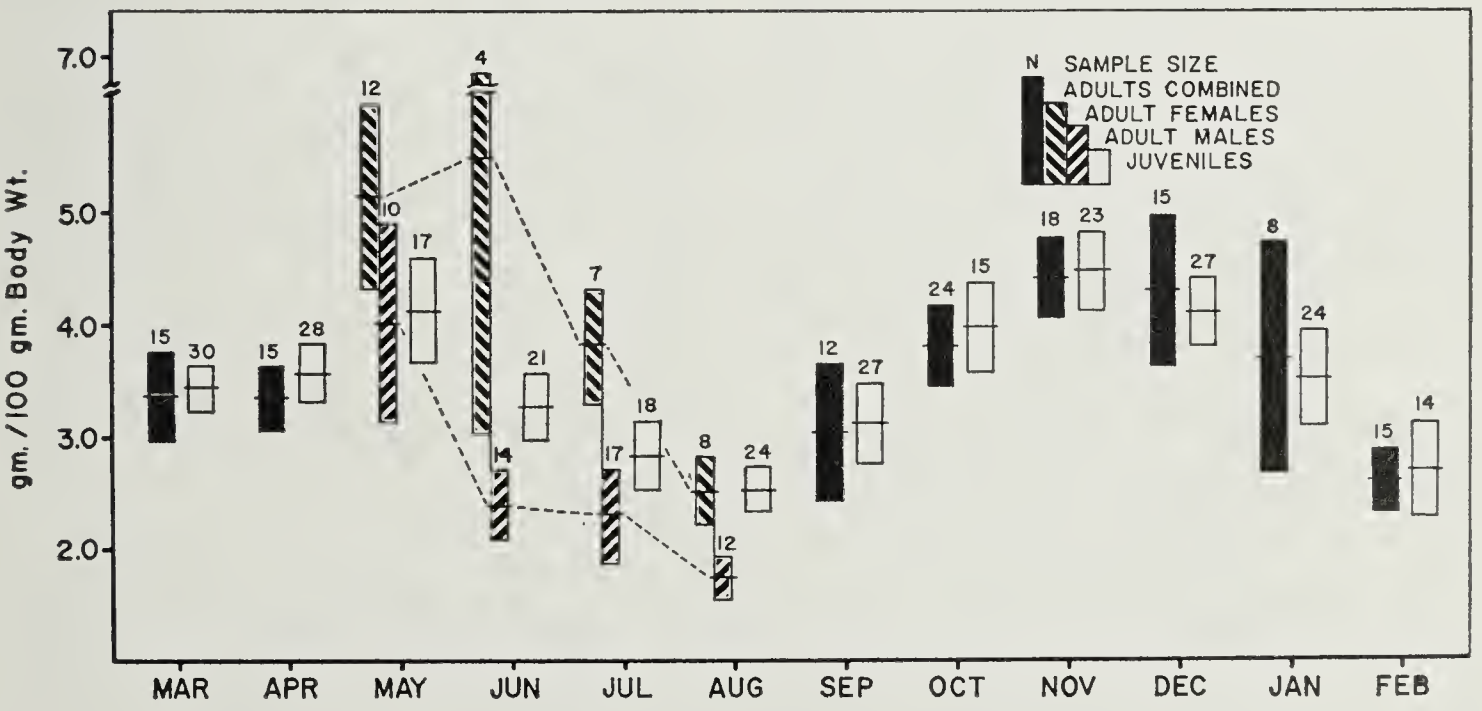

FIGURE 3.—Mean monthly liver weights (expressed as g/100 g eviscerated body weight) of 474 green frogs collected March 1963 to February 1964 in southern Illinois. The rectangular boxes delineate the 95% confidence limits of each class per month. The horizontal line through each box indicates the mean.

Liver. Mean monthly liver weights for all classes of collected green frogs alternated between two maxima (May, November) and two minima (August, February) values during the year; these extremes in liver weight fluctuations occurred during the last month of each 3-month season (Fig. 3). Frogs taken in May and November had the heaviest monthly liver weights, averaging 4.34 and 4.50 g/100 g body weight, respectively. Mean monthly liver weights of August frogs (2.29 g/100 g body weight) and February frogs (2.59 g/100 g body weight) were the lightest of the year (Fig. 3).

In contrast to the annual liver weight cycle in green frogs, Smith (1950) working with *Rana temporaria* and Bush (1963) using *Bufo fowleri* found liver weights to decrease during the spring instead of increase as seen in *R. clamitans*. Liver glycogen content in *R. temporaria* (Smith, 1950), *B. fowleri* (Bush, 1963) and *Rana pipiens* (Mizell, 1965) was also shown to be less at this time of year. A characteristic these three anurans have in common, which is not shared with *R. clamitans*, is a late winter or spring breeding season. *Rana clamitans* is feeding heavily during the spring and not breeding.

Through the summer months (June-August), liver weights of green frogs progressively decreased to a degree not observed in *R. temporaria* (Smith, 1950), *B. fowleri* (Bush, 1963), and *Bufo arenarum* (Houssay, 1949). August marked the lowest mean liver weights in all classes of green frogs for the year (Table 1). With the possible exception of adult males, a lack of food did not appear to be responsible for liver atrophy in the summer green frogs (Fig. 1). The ambient temperature, however, may have been a contributing factor to the observed decrease in liver weight as the rising summer temperatures (Fig. 2) probably increased the metabolic activity of these ectotherms (Cook, 1949; Vinegar and Hutchison, 1965). Mizell (1965) found the quantity of liver glycogen in *R. pipiens* to be less during the summer than at any other season of the year. Smith (1954) reported that excitement in spring and summer *R. temporaria* can cause marked hyperglycemia. For the summer green frog, then, the energy demands of a long breeding season, higher ambient temperatures, and accelerated interactions with the environment would very likely contribute to decreased liver weight.

In September, liver weights of

Table 1.—Sample size (N), range, mean ($\bar{u}$), and standard deviation (S.D.) of liver weights (expressed as g/100 g eviscerated body weight) of 474 green frogs collected from March 1963 to February 1964 in southern Illinois.

Month	Juvenile			Adult Male			Adult Female		
	N	Range	$\bar{u}\pm$S.D.	N	Range	$\bar{u}\pm$S.D.	N	Range	$\bar{u}\pm$S.D.
Mar.	30	2.34-5.04	3.41±0.56	8	2.61-4.10	3.17±0.48	7	2.88-5.12	3.55±0.73
Apr.	28	2.25-5.05	3.55±0.66	11	2.73-3.63	3.28±0.29	4	2.14-4.33	3.73±0.98
May	17	2.76-5.73	4.07±0.88	10	1.63-6.12	3.95±1.18	12	2.25-7.04	5.04±1.31
Jun.	21	2.49-4.50	3.26±0.65	14	1.65-3.20	2.36±0.53	4	4.80-6.80	5.41±0.93
Jul.	18	1.84-4.00	2.85±0.56	17	1.13-3.84	2.24±0.82	7	2.90-4.44	3.73±0.53
Aug.	24	1.68-3.43	2.51±0.49	12	1.28-2.20	1.72±0.29	8	1.89-2.93	2.50±0.36
Sep.	27	1.93-5.48	3.06±0.91	5	1.68-4.26	2.92±1.01	7	2.14-4.91	3.05±0.95
Oct.	15	2.91-5.48	3.92±0.74	12	2.09-4.33	3.34±0.60	12	2.39-5.36	4.15±0.82
Nov.	23	2.67-6.16	4.43±0.83	9	3.45-4.93	4.32±0.56	9	3.31-5.64	4.41±0.90
Dec.	27	2.39-5.84	4.05±0.79	6	2.74-6.02	4.62±1.22	9	2.35-5.59	3.97±1.09
Jan.	24	2.00-6.39	3.47±1.05	2	2.36-2.40	2.38±0.03	6	2.98-5.36	4.04±1.17
Feb.	14	1.81-4.24	2.66±0.73	8	2.15-3.28	2.47±0.36	7	1.62-3.53	2.72±0.63

green frogs began to increase, raising in November to their greatest mean value of the year. Similar fall increases in liver weights are reported for *R. temporaria* (Smith, 1950) and *B. fowleri* (Bush, 1963) as is increased liver glycogen content in *R. temporaria* (Smith, 1950), *R. pipiens* (Mizell, 1965), *B. fowleri* (Bush, 1963) and *B. arenarum* (Mazzocco, from Bush 1963).

During November, the green frogs became increasingly scarce as they entered hibernacula; this behavior was probably in response to environmental temperatures dropping below the species' voluntary minimum temperature tolerance of 50 F (Brattstrom, 1963). Martof (1956a) found the initiation of hibernation in Michigan green frogs was correlated with air temperatures similar to those which existed at Pine Hills in November (Fig. 2).

After November, liver weights progressively decreased through February,when the lowest mean monthly liver weight of the winter occurred. Ingestion of food was at a minimum from December through February (Fig. 1). Undoubtedly this three month period of partial inanition had a decided effect upon liver weights. Starvation has been shown to dramatically decrease the size of livers of *Rana fusca* (Jackson, 1929) and *R. pipiens* (Ott, 1924).

Though no statistical differences were noted between the sexes of juvenile frogs, mean monthly liver weights of adult males were less than those of adult females; the only exception in the data occurred in December (Table 1). March (1937) and Smith (1950) found a similar trend in *R. temporaria*. However, unlike the differences reported for male and female *R. temporaria,* liver weight differences between green frog adult sexes were statistically significant ($P=<0.01$) for June, July, and August (Fig. 3).

The liver weights of adult males during the summer (June-August) were significantly less ($P=<0.02$) than corresponding values for the other two classes of frogs, and in August, livers of adult males fell to the smallest monthly value (1.72 g/100 g body weight) for any group during the year. Adult males ingested far less food during June through August than either adult females or juveniles (Fig. 1). It is during these summer months that the males are sexually active, establishing territories (Martof, 1953; Oldham, 1967), and apparently defending these territories against other male green frogs (Jenssen and Preston, 1968; Schroeder, 1968).

Fat Bodies. The fat body weights of the green frogs were extremely variable within any one month, but did seem to follow a general trend for the year (Fig. 4). For all classes of frogs there was a similar biannual rise and fall of fat bodies weights as occurred with the green frogs' liver weights. February frogs averaged the lightest fat bodies of the 3-month winter season, May frogs had the heaviest mean fat body weights of the spring months, both July and August frogs contained little or no fat bodies, and then the November frogs had the heaviest mean fat body weights of the 3-month fall season. Each of these extreme seasonal oscillations in mean monthly fat body weights was significantly different ($P=<0.01$) from adjacent seasonal maximum or minimum mean monthly values.

The light fat bodies and livers of summer juvenile green frogs suggest that factors other than those unique to adults (i.e. low food intake of territorial males and egg production in females) are responsible for major energy expenditures. Then in September, the recruitment of many newly metamorphosed frogs into the juvenile class caused a sharp rise in the average juvenile fat body weight (Fig.

TABLE 2.—Sample size (N), range, mean (ū), and standard deviation (S.D.) of fat body weights (expresses as g/100 g eviscerated body weight) of 474 green frogs collected from March 1963 to February 1964 in southern Illinois.

Month	Juvenile			Adult Male			Adult Female		
	N	Range	ū±S.D.	N	Range	ū±S.D.	N	Range	ū±S.D.
Mar.	30	0.02-0.49	0.11±0.56	8	0.01-0.86	0.22±0.30	7	0.00-0.46	0.13±0.16
Apr.	28	0.00-1.01	0.09±0.19	11	0.00-0.19	0.08±0.07	4	0.00-0.22	0.07±0.10
May	17	0.02-1.76	0.52±0.55	10	0.00-1.62	0.55±0.59	12	0.03-2.23	0.62±0.65
Jun.	21	0.00-0.54	0.15±0.13	14	0.00-1.03	0.43±0.36	4	0.00-2.20	0.61±1.07
Jul.	18	0.01-0.10	0.04±0.02	17	0.00-0.03	0.01±0.01	7	0.00-0.03	0.02±0.01
Aug.	24	0.00-0.31	0.05±0.07	12	0.00-0.06	0.01±0.02	8	0.00-0.02	0.01±0.01
Sep.	27	0.00-2.87	0.25±0.60	5	0.01-0.06	0.02±0.02	7	0.00-0.06	0.02±0.02
Oct.	15	0.00-0.31	0.12±0.11	12	0.02-0.20	0.08±0.07	12	0.02-0.63	0.23±0.19
Nov.	23	0.01-1.69	0.34±0.42	9	0.03-1.76	0.61±0.56	9	0.00-1.24	0.50±0.54
Dec.	27	0.04-0.41	0.17±0.11	6	0.03-0.89	0.47±0.33	9	0.02-0.56	0.24±0.22
Jan.	24	0.00-1.46	0.13±0.29	2	0.02-0.16	0.09±0.10	6	0.00-1.98	0.60±0.75
Feb.	14	0.00-0.14	0.04±0.04	8	0.00-0.12	0.04±0.05	7	0.00-0.31	0.09±0.14

4). These young frogs all had large fat deposits which presumably sustain them during their metamorphic fasting period. Because of the still small fat bodies of older juvenile frogs, a wide range of fat body weights resulted for the September juveniles (Table 2).

In comparison with some other species of anurans, seasonal fat body fluctuations in the green frog differed distinctly. Fat bodies diminished during the spring in *R. pipiens* (Mizell, 1965), *R. temporaria* (Smith, 1950), *B. fowleri* (Bush, 1963), and *B. arenarum* (Houssay, 1949), while *R. clamitans* had enlarged fat bodies at this time of year. Here again, the life history characteristics of the green frog are different from the above species. The variation in the annual fat body weight cycle of these species may reflect the difference in breeding periods, as fat body size appears inversely correlated with egg production (Anderson, 1954; Brenner, 1969). It is suspected that other anuran species (e.g. *Rana catesbeiana*) with prolonged summer breeding seasons would also experience a biannual expansion of fat bodies.

Reproductive Organs. There was a consistent weight difference between the paired reproductive structures of the green frog. The left gonad was heavier than the right (Table 3); of the 456 pairs of gonad weights determined, only 6 pairs (1.3%) were exceptions .Miller and Robbins (1954) also found a weight difference between the paired testes in the salamander, *Taricha torosa.* However, they found the right testes to be heavier than the left; their report also cited other studies which found the same condition in other salamanders. The Javanese toad, *Bufo melanostictus,* occupies an intermediate condition, having no observed difference occurring between the weights of its gonads (Church, 1960).

During May, adult green frog ovaries contain mature or almost mature oocytes which were much larger than those found in April frogs. Ovaries from June females reached their heaviest weights of the year (Fig. 4); the left and right ovaries averaged 10.38 and 6.81 g/100 g body weight, respectively. While studying *R. clamitans* in Michigan, Martof (1956b) first discovered egg masses deposited on May 31. Inspection of ovaries from frogs of the present study indicated eggs were not laid earlier than May in southern Illinois, with peak

TABLE 3.—Sample size (N), range, mean ($\bar{u}$), and standard deviation (S.D.) of gonad and oviduct weights (expressed as g/100 g eviscerated body weight) of 204 adult green frogs collected from March 1963 to February 1964 in southern Illinois.

Organs	March	April	May	June	July	August	September	October	November	December	January	February
Adult Testes												
Right												
N	8	11	10	14	17	12	5	12	9	6	2	8
Range	0.06—0.11	0.05—0.13	0.11—0.20	0.12—0.26	0.05—0.23	0.09—0.22	0.07—0.13	0.04—0.16	0.12—0.14	0.08—0.11	0.08—0.10	0.06—0.13
$\bar{u}\pm$S.D.	0.08±0.02	0.09±0.03	0.15±0.04	0.18±0.04	0.16±0.04	0.14±0.05	0.10±0.02	0.11±0.03	0.13±0.01	0.10±0.01	0.09±0.02	0.10±0.02
Left												
N	8	11	10	14	17	12	5	12	9	6	2	8
Range	0.08—0.14	0.10—0.18	0.14—0.31	0.13—0.28	0.14—0.32	0.12—0.25	0.09—0.16	0.05—0.22	0.11—0.22	0.09—0.14	0.09—0.11	0.10—0.15
$\bar{u}\pm$S.D.	0.11±0.03	0.14±0.03	0.20±0.06	0.22±0.04	0.22±0.05	0.17±0.05	0.14±0.03	0.14±0.05	0.14±0.05	0.12±0.02	0.10±0.01	0.12±0.02
Adult Ovaries												
Right												
N	7	4	12	4	7	8	6	11	9	9	6	7
Range	0.51—0.99	0.61—1.06	0.77—5.87	3.34—11.55	0.75—8.76	0.25—0.69	0.34—0.52	0.38—0.99	0.37—1.10	0.53—3.04	0.60—1.30	0.62—3.25
$\bar{u}\pm$S.D.	0.72±0.16	0.80±0.20	1.65±1.39	6.81±3.52	3.72±2.89	0.51±0.16	0.45±0.07	0.61±0.20	0.66±0.22	0.95±0.80	0.88±0.25	1.30±1.02
Left												
N	7	4	12	4	7	8	6	11	9	9	6	7
Range	0.73—1.35	1.01—1.58	0.92—9.31	4.87—16.53	0.77—12.15	0.43—1.08	0.41—0.72	0.52—1.20	0.48—1.20	0.59—3.47	0.73—1.80	—0.74—3.54
$\bar{u}\pm$S.D.	0.96±0.21	1.19±0.26	2.27±2.25	10.38±5.48	5.22±3.82	0.66±0.22	0.60±0.15	0.76±0.20	0.86±0.21	1.19±0.89	1.18±0.39	1.57±1.15
Adult Oviducts												
Right												
N					7	8	6	11	9	9	6	7
Range					0.70—6.05	0.06—0.57	0.01—0.42	0.09—0.59	0.11—0.82	0.10—2.20	0.11—0.68	0.09—1.57
$\bar{u}\pm$S.D.					2.40±1.75	0.42±0.19	0.15±0.14	0.30±0.19	0.31±0.21	0.43±0.67	0.44±0.24	0.53±0.54
Left												
N				4	7	8	6	11	9	9	6	7
Range				1.85—5.02	0.55—5.85	0.06—0.57	0.02—0.38	0.09—0.57	0.10—0.70	0.08—1.96	0.10—0.73	0.09—1.40
$\bar{u}\pm$S.D.				3.26±1.48	2.25±1.87	0.40±0.19	0.14±0.13	0.28±0.19	0.27±0.19	0.38±0.60	0.44±0.26	0.50±0.48

readiness during June. By July, adult females were prepared to ovulate or were already spent, as reflected in the large range of ovarian weights for July (Table 3). All females collected in August had ovulated and presumably the breeding season was over; the adult males, however, continued to call throughout August.

Martof (1956a) states the possibility that not all gravid females lay each year, and this may well be the case for the Pine Hills population. One gravid female was collected each month during September and October from LaRue Swamp, and these were

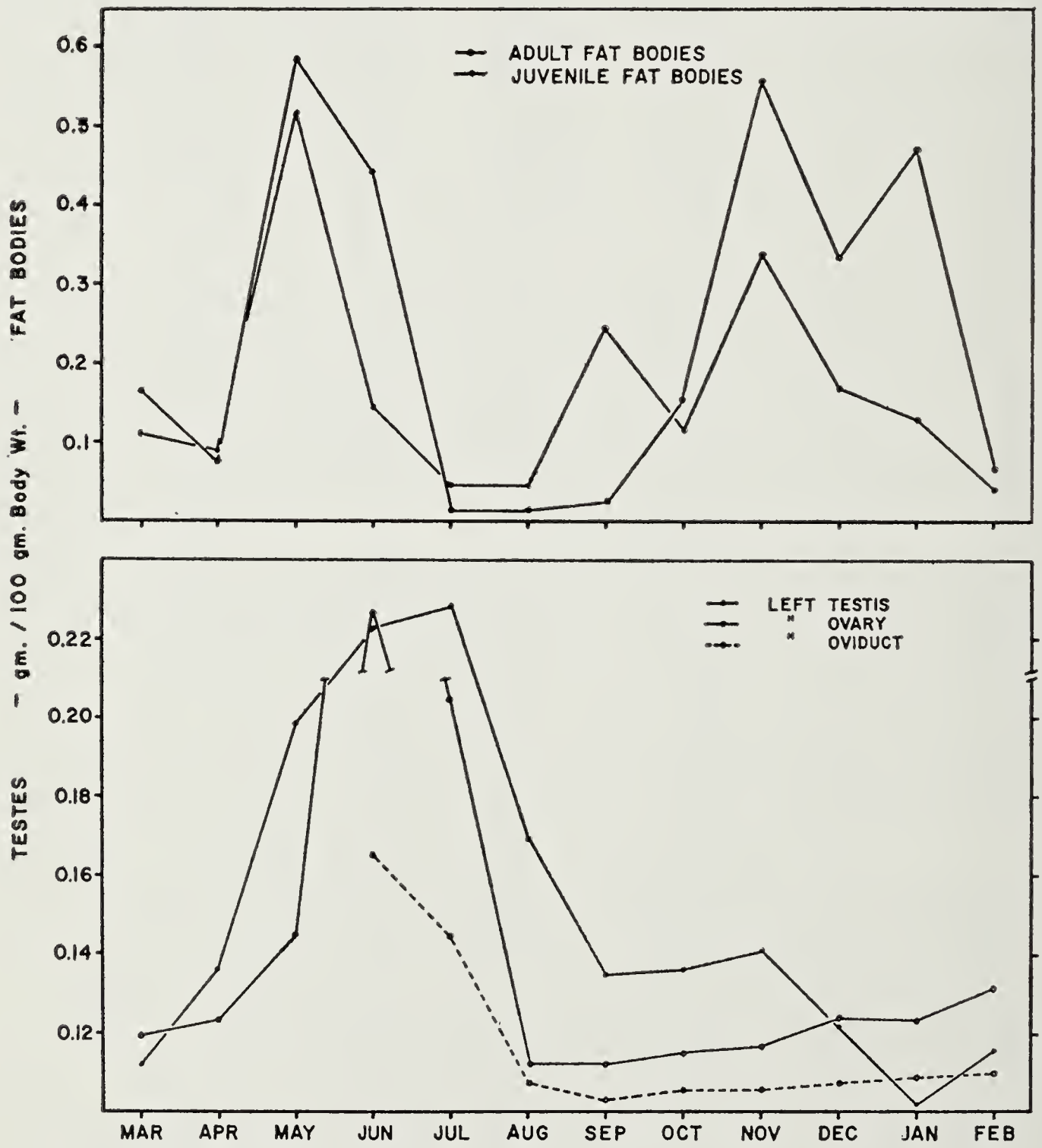

FIGURE 4.—Mean monthly weights of fat bodies and adult reproductive structures (expressed as g/100 g eviscerated body weight) for 474 and 204 frogs, respectively, from a sample of 474 green frogs collected March 1963 to February 1964 in southern Illinois.

the only females during the fall months whose ovarian weights exceeded 1.2 g/100 g body weight. The left ovaries of the gravid September and October females weighed 3.58 and 8.61 g/100 g body weight, respectively. Because these adults were considered anomalies, their gonad and oviduct weights were deleted from the data presentation.

The sizes of the paired oviducts in the green frogs were also asymmetrical In contrast to the ovaries in which the left was usually the heavier of the pair, the right oviduct was heavier than the left; this was true of 80 percent of the adult females examined.

Oviduct weights of adult frogs collected from June to February followed the same weight changes as the ovaries (Fig. 4). Though no data were obtained during March through May, it is probable that the annual weight fluctuations of the oviduct closely approximate the ovarian weight cycle. This weight relationship between oviduct and ovary has been found in *Microhyla carolinensis, Xenopus laevis, R. temporaria, R. pipiens* (Anderson, 1954; Smith, 1955).

Testicular weights of adults rapidly increased in April, coinciding with the commencement of calling, and reached maximum values during June and July when adult females were found to be in reproductive readiness. Testes of the adult males decreased in weight during August, the last month of calling. The testes enlarged a month before the ovaries and did not show a substantial weight loss until a month after collected females were found spent. The male sexual period, then, overlaps the egg laying interval, thus insuring efficient fertilization of females.

In spring breeding anurans, spermiogenesis usually occurs in late winter or early spring, following a resting period during fall and early winter; spermatogenesis and testicular proliferation take place after the breeding period during late spring and summer (Oslund, 1929; van Oordt and van Oordt, 1955; van Oordt et al., 1959). Thus in *R. pipiens* (Glass and Rugh, 1944; Mizell, 1964), *R. temporaria* (Smith, 1950; van Oordt, 1956), and *B. arenarum* (Mazzocco, from Bush, 1963), maximum increase of testicular weight comes after the breeding season when spermatogenesis occurs. However, such a seasonal weight cycle was not observed for *R. clamitans.* Maximum weight of testes occurred during the breeding season. These observations deserve further investigation and will likely show that spermatogensis is maintained during the long breeding period of the green frog, in contrast to short-term breeders.

Acknowledgments

Gratitude is extended to Dr. W. D. Klimstra who made this study possible; it is a contribution from Project No. 10, Cooperative Wildlife Research Laboratory, Southern Illinois University.

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Manuscript received February 26, 1971.

AN ILLINOIS SCIENTIST DEFENDS DARWINISM: A CASE STUDY IN THE DIFFUSION OF SCIENTIFIC THEORY

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ABSTRACT.—This article is a study of the remarks made by Charles V. Riley, an entomologist who began his career in Illinois, and Dr. George Engelmann and Judge Nathaniel Holmes, members of the Academy of Science of St. Louis, over the theory of evolution. The time period is 1871-1877. The author seeks to show how gradually the theory was accepted or rejected by educated men of the period. All quotations are from the "Journal of Proceedings," Academy of Science of St. Louis, *Transactions,* 3: i-cclxxv.

Everyone knows that Asa Gray defended Darwin and the theory of evolution against Louis Agassiz. At the same time and later, lesser skirmishes also went on in the United States. Exchanges of views in the years 1871 to 1877 took place between Charles V. Riley, an entomologist who got his start in Illinois, and two members of the Academy of Science of St. Louis. The dates themselves are significant because they show that discussion of *The Origin of Species,* published in 1859, among intellectuals such as doctors, lawyers, and others who had an interest in science in an amateur or semi-professional way, was long delayed. This was true not only in St. Louis, but in other cities of the Middle West as shown in sparse mention of evolution in the minutes and proceedings of other nineteenth century academies of science and natural history societies which the author has examined under a grant from the National Science Foundation.

Charles V. Riley the man who bore the banner of evolution in St. Louis was born in England in 1843. He was educated in France and Switzerland, where, as a boy, he learned natural science, being particularly interested in the insect world. Gifted as an artist, he augmented his collecting activities by drawing elegant pictures of his specimens. He came to the United States when he was seventeen years old, and found work on a farm near Chicago. He continued his interest in entomology, and educated himself by intensive reading and by observing the habits of insects in the course of his farm labors. His drawings attracted the attention of the editors of *The Prairie Farmer* a Chicago journal that printed material on natural science as it affected agriculture and they accepted some of Riley's studies of insects. The youth enlisted in the Union army during the Civil War, and after his military service, he resumed his career in Chicago as a reporter and artist for *The Prairie Farmer.* Later he served briefly under Benjamin D. Walsh, the first State Entomologist, and then moved to Missouri to become State Entomologist, and made his headquarters in St. Louis where he was a member of the St. Louis Academy of Science, serving as president and secretary (Packard, 1895; Howard, 1935).

The Academy of Science was founded by Dr. George Engelmann and his friends to provide a forum for the exchange of scientific information, the maintenance of a museum, and the publication of a scientific journal (Hendrickson 1958, 1966). Dr. Engelmann, a distinguished botanist, as well as a practicing physician was

born in Germany in 1809, and was educated there and in France. He received his M.D. degree from the University of Würzburg. Throughout his school years, as well as later in life, he spent as much of his time in the study of natural history as he did at his profession. Engelmann corresponded with and exchanged specimens with botanists and museum curators in Europe and America, and he was recognized as an authority on western plants and trees (Bek, 1929).

In 1871 Riley and Engelmann confronted each other over the validity of the views of Charles Darwin. Engelmann, European educated, and secure in his place as a leading botanist, was concerned but little with speculative or philosophical aspects of science; Riley, self-educated, half the age of the doctor, on his way up in a developing scientific field, was much interested in scientific theory. The first encounter took place at the meeting of the Academy of Science on March 6, when Engelmann in his usual dry manner, exhibited three specimens of Indian mallow, a common weed, which he said were being used for papermaking. One specimen was from France, the second from Italy, and the third from St. Louis County in Missouri. Engelmann pointed out that there was very little difference in the appearance of the three plants. Then Riley dropped a bombshell whose fragments continued in the air for years. He asked if "Dr. Engelmann had any explanation for the fact that so many of our common plants and seeds were introduced from abroad, and had been naturalized in America." Riley declared that his studies had shown that dozens of injurious insects were of European origin, "but only two or three American species had ever greatly multiplied [in Europe]."

Dr. Engelmann replied that some plants were more vigorous than others, but he felt that the point was not important since he was "disinclined to believe that there was such contrast as Riley pictured." Riley, however, would not be silenced, and said that he "undertook to account [for the phenomenon] by the Darwinian theory, that from the greater competition and struggle for existence that had gone on in Europe under the civilized conditions of man, their species were, many of them, better able to thrive under similar conditions here than our own indigenous species."

Utterly unconvinced that the struggle for existence had any validity, Engelmann replied that "he feared such theories would lead us astray." The good doctor was supported by his friend, Dr. Adolph Wislezenus, who had much the same kind of European education. The latter said "that the greatest increase in European plants when introduced to this country was due to the fact that they had more room here than in their native soil."

Engelmann himself refused to discuss the matter further and stayed silent, when, on April 7, 1871, Riley returned to the idea of the survival of the fittest, and brought up the subject of mimicry and protective resemblance in animals. He used as an example the mimicry of Monarch butterflies by Viceroys, saying that this was a case of the resemblance of one species that was edible to birds [Viceroy], to another which was not edible [Monarch], with the latter thus being protected. This, Riley said, was a case of natural selection such as Darwin had described in *The Origin of Species*.

It was almost a year (December 16, 1872) before Riley brought up the matter again. This time it was in the midst of his remarks when he presented to the Academy the skin of a six-foot rattlesnake with twenty-eight rat-

tles attached. Riley said, "The rattles of the rattlesnake had been a stumbling block to the Darwinians, it is generally taken for granted that they must prove a serious detriment to the snake by frightening away any prey that might come within reach." Riley noted, however. that some naturalists held that the noise of rattling attracted birds, because it resembled the cicada, and others declared that the snake did not sound its rattles except when it wanted to scare an enemy. "Or," he said in conclusion, "if this view proved unsatisfactory, this explanation would serve: that the rattle served to call the sexes together, [which] would still prove its advantage to the snake." There was no comment from Engelmann or others.

Now another member, Judge Nathaniel Holmes, joined in the discussion. Holmes was born in Peterborough, New Hampshire in 1815, and he was a graduate of Harvard College and Harvard Law School. He came to St. Louis in 1839, where he had a notable career as a lawyer and a judge. Among his many interests were archeology and anthropology, and he was an early participant in the excavation of Indian mounds in the St. Louis area (*National Cyclopedia of American Biography,* 1893.) Holmes was the corresponding secretary of the St. Louis Academy of Science, and he saw to it that the other members kept up with the progress of science by reading to them, or summarizing for them, articles that he found in the publications received by the Academy in exchange for its *Transactions.* One evening the judge read an article describing the discovery in the Mentone caves of France of the fossil remains of man in association with bones of mammoths and cave bears. He remarked that "twenty years ago. geologists did not admit that man was older than the recent alluvial deposits. But geologists will have to trace these human remains still further back, and even into the Miocene period, before they arrive at the anthropoid Dryopithecus of that age, which Mr. Darwin suggests [in *The Descent of Man,* 1871] as indicators o fthe point of transition, in the course of the linear branching descent, from the anthropoid to the completely human type." It was Holmes' conclusion: "What objections can there be to that; if it pleased the Creator to take that length of time for His work and to do it in that way?"

Neither Engelmann nor Riley had any comments to make, but a few months later, on November 5, 1873, when Holmes read a paper that said that remains of man were found in strata as old as the Triassic, Engelmann spoke up, saying that he could not believe that man lived in such an early age, any more than he could accept proofs that were given "to establish the theory of evolution of man out of the apes." On April 6, 1874, Holmes called attention to the famous article by Othniel C. Marsh in *The American Journal of Science* in which the Yale paleontologist demonstrated the validity of evolution by the decreasing number of toes in the feet of fossil horses. Holmes commented that Marsh's study seemed to prove "that while it is plain that the change from the Cretaceous 5-toed vertebrate involved some kind of continuation, the problem was to get a conception of the mode and manner of that continuation." Continuous descent from father to son—from individual to individual was an erroneous conception. Holmes continued, "Descent is only from the pair, and there is an interweaving of pairs until all are related." Holmes thought that "this view presents us with the true conception of the doctrine of evolution; and so conceived, that doctrine would cease to be objectionable. Such

a conception is still in harmony with the idea of new creation and also of evolution."

Riley objected vigorously from Holmes ontological views, saying that "the process of evolution through climatic change, natural selection, and any other natural differences now recognized by evolutionists, were sufficient to account for the origin of species, and that to conceive of the modification through any other means then descent was begging the question, and it was only another way of expressing the old ideas of special creation."

Holmes and Riley tangled again when the latter told about the rank growth of grass which was immune to attack by locusts. "as a beautiful illustration of what Darwin called 'the struggle for existence.'" Holmes "objected to the term 'struggle for existence' as if a plant could struggle. If it was intended literally, either it is asserted that a plant thinks and wills, or it is asserted than an animal possessing will struggles only in the same way." Holmes ended his remarks by saying that he "could not help feeling a little indignation at Mr. Darwin's illogical use of such expressions as 'natural selection' or 'a law acting' as if those things could do anything. A law does not act; it is merely operative by sheer obstruction and resistance. It is not an active power."

Riley patiently explained to Holmes that "natural selection" and "a law acting" as Darwin used them. did not imply conscious action and that "struggle for existence" should no more be taken in its literal sense than any other expression that had "come to have a special meaning by common acceptance and accord." Holmes, on December 4, 1876, still refusing to be convinced of the validity of Darwinism, returned to the subject, and "criticized Mr. Darwin and some of his supporters for ignoring the cause of evolution, and confining their attention to the laws according to which evolution takes place." Riley again tried to straighten out Holmes' thinking by pointing out that "neither Mr. Darwin, nor many of his supporters, denied the existence of the cause, but simply declined to discuss this point, which appeared to them to be sufficient." Whether Nathaniel Holmes ever accepted natural selection is not known, because soon after this he retired from law practice and returned to Cambridge, Massachusetts.

But Riley and Engelmann were growing closer together in their thinking. At a meeting on March 20, 1876, Dr. Engelmann talked about the classification of oak trees, and in the course of his presentation, he remarked that *Quercus densiflora* of California "may be hybrid between the chestnut and the oak in bygone time when these genera were less differentiated." and, he continued, "it is well-known that even now hybrid oaks are not sterile like most hybrids, but will propagate when removed from 'the struggle for existence' against more hardy rivals." Riley immediately tried to press the point that the facts cited by Engelmann "would indicate a common origin in the past for the two kinds of trees." But Engelmann avoided a commitment, saying that "the oak and chestnut of Tertiary times [the geological age in which he had said that probably oaks had lobed leaves like those of chestnuts] appear to be distinct as they are today."

The young entomologist had no such doubts about Darwin and the origin of species as did the dogmatic Holmes or the reluctant Engelmann, and he had an opportunity to state his unqualified support of evolution when he made his presidential address to the St. Louis Academy on January 15, 1877. As was the custom, the president reviewed the developments in science during the past year,

and Riley included a section on evolution, mentioning the work of Asa Gray and Thomas Huxley in persuading people to accept evolution.

Riley was the first president of the Academy to bring up the matter, and he said that "all great truths that oppose long established popular belief must need to belong to the few when nascent. Struggling to overcome the embargo which prejudice and ignorance always set in their path, they at last win acceptance from the mass of thinking men, who by that time wonder how there could ever have been serious objections to the new light." Riley concluded by saying that "in my humble opinion, the idea of evolution is founded on fact, and, like a gem freed from the deposits which for ages have hidden its lustre, will shine all the brighter as the obstacles which surround it are removed by the light of truth."

But what of Dr. Engelmann, who had sat silent for the most part during the six years that Riley brought up again and again the worrisome matter of evolution. Finally, as though to demonstrate Riley's confidence that truth will prevail, Engelmann addressed the Academy on November 19, 1877, returning to the subject that had started the long debate, the geographical distribution of North American flora, by describing the introduction of plants into North America by colonists from England, and into the Southwest by Spaniards, and then noted that seeds came over in various ways aboard ships.

As he concluded his remarks, he said, "A recent means of extending the area is through cattle drives from the Southwest, and especially through the extension of the railroads . . .; such foreign plants often take hold of extensive grounds as the fittest to survive." It was a small quiet voice, but Dr. Engelmann's conversion was a vindication of Charles Riley's long defense of evolution.

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Manuscript received January 12, 1972

PHOTOCHEMICAL REDUCTION BY ISOLATED CHLOROPLASTS OF A SOYBEAN MUTANT

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ABSTRACT.—*Glycine max* with a nuclear mutation that segregates in regard to chlorophyll content was utilized to conduct a comparative study of photochemical reduction by isolated chloroplasts. Photochemical reduction by isolated chloroplasts of the double recessive lethal mutant was shown to be higher than that of the heterozygous mutant or wild type when expressed on a per chlorophyll basis.

Kirk and Tilney-Basset (1967) have described many higher plants which have a depleted chlorophyll content. Highkin, Boardman, and Goodchild (1966, 1967, 1969), using a chlorophyll depleted pea mutant, have shown that more light is required for saturation and that photoreduction of trichlorophenolindophenol (TCIP) occured at four times the rate of the wild type plant. A *Glycine max* mutant described by Weber and Weiss (1959) was used in our study. This mutant, in which a one gene nuclear mutation expresses incomplete dominance as measured by chlorophyll content, yields three phenotypes. The wild type homozygote or dark green (DG), the heterozygote which are light green (LG), and the lethal yellow (LY) homozygote all occur in a 1.2:1 phenotypic and genotypic ratio. Photosynthetic carbon dioxide uptake of LG plants was shown by Wolf (1965) to saturate at the same light intensity as the DG plants. Light saturation was also found to occur at the same intensity for photosystem I and II in both the LG and DG plants by Keck, Dilley, and Ké (1970). A model for the light reactions of photosynthesis appears in Figure 1. This figure is an adaptation from Salisbury and Ross (1969) and illustrates photosystems I and II. The chloroplast composition and ultrastructure of these genotypes was also determined and led to the suggestion of a correlation between chloroplast biochemistry and membrane ultrastructure by Keck, Dilley, and Ké (1970). They found a 3-5 fold increase in the electron transport rate of the LG plastids over the DG plastids and proposed that the chloroplasts of the mutant have a significantly faster turnover time for plastoquinone oxidation, which has previously been identified as a rate limiting reaction for electron transport. We have measured photochemical reduction by isolated chloroplasts of all three genotypes, and present further evidence for Keck, Dilley, and Ké's proposal by including data for the homozygous lethal mutant, LY.

METHODS AND MATERIALS

The experimental plant material was grown in a Percival growth chamber in flats containing a soil-vermiculite mixture. The chamber was timed to a 12:12 hour light: dark cycle. Light temperature was maintained at 29° C and dark temperature at 20° C. Illumination at leaf level was 400 ft-c. The low light level enabled the LY plants to survive for as long as two months. Humidity ranged from 40-60%.

Plant tissue was harvested from ten-day-old seedlings. Only expanded uni-

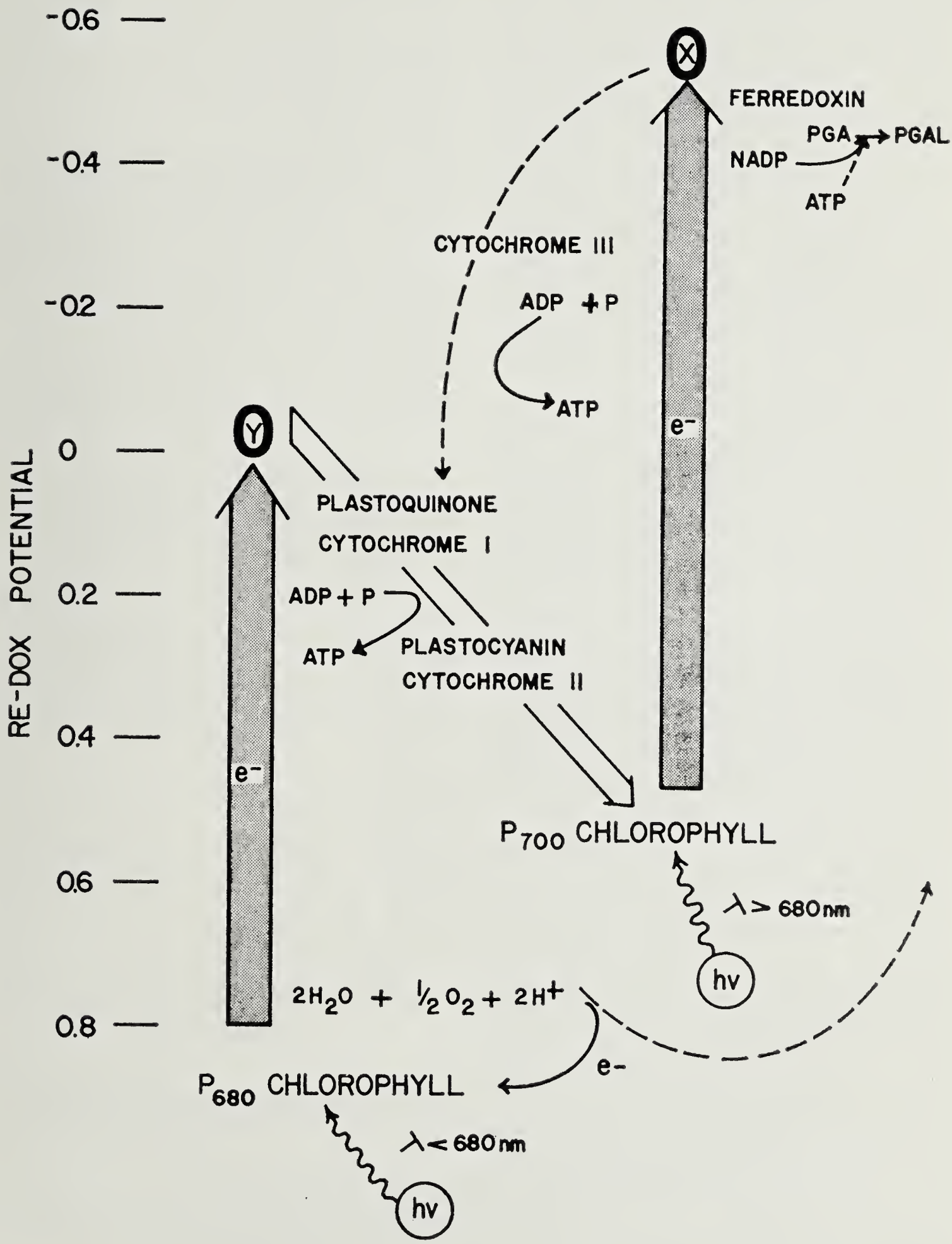

FIGURE 1.—Proposed model of photosystems I and II. Under experimental conditions DPIP is the final electron acceptor.

foliate leaves were used. The plant tissue was macerated with a cold mortar and pestle, and the chloroplasts isolated in cold 0.35M sodium chloride according to the procedure of Dunn and Arditti (1968). The final chloroplast preparation was resuspended in cold 0.35 M NaCl. Total chlorophyll concentration was determined by the method of Arnon (1949) using the isosbestic point of chlorophylls a and b (652 nm). The chlorophyll content of the isolated chloroplasts of the three genotypes is shown in Table 1.

Two sets of reactions were performed to determine the relative rates of photoreduction exhibited by isolated chloroplasts of the three genotypes. Both sets of reactions utilized the dye 2, 6 - dichlorophenolindophenol (DPIP), 2.4 x 10^{-5} M, as the Hill reductant.

The first set of reaction mixtures contained: 1.0 ml phosphate buffer (pH 8.0), 1.0 ml of chloroplast suspension containing 4.0 ug of chlorophyll, 1.0 ml of 1.2 x 10^{-4} M DPIP solution, and 2.0 ml of distilled water. The reaction vessels were placed in an iced water bath (5° C) and illuminated with a 500 watt flood lamp 12 inches from the reaction vessels which, as shown by preliminary experiments, provided a light intensity above the light saturation level of the reaction. Absorbance readings were taken at five minute intervals on a Bausch and Lomb Spectronic 20 to determine the amount of dye reduced. In order to eliminate the effect of plastids in the suspension of the measurable amount of dye reduced, the reaction vessels were centrifuged at 2000 x G for 7 minutes. The time curves, expressed on a per unit chlorophyll basis, are shown in Figure 2.

The second set of reaction mixtures was prepared in the same manner with the following changes: The LG vessel received 30 ug of chlorophyll and the DG vessel received 80 ug of chlorophyll. All of the reaction vessels contained 3.0 ml DPIP. Procedures followed during this set of reaction runs were the same as those described for the first set of reactions.

Results and Discussion

The time curves plotted from the results of this set of reactions were

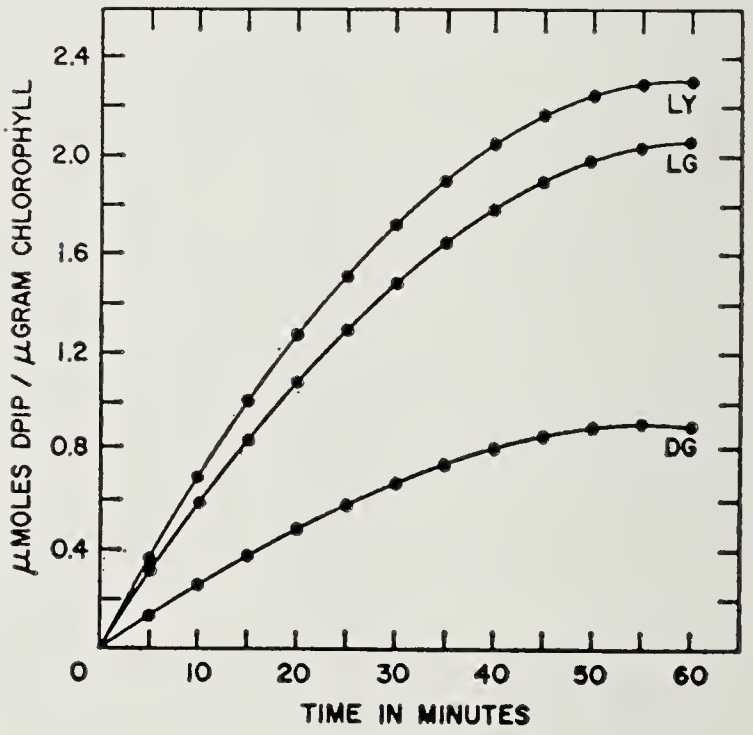

Figure 2.—Plot of umoles DPIP reduced per ug chlorophyll from each genotype.

MGS. CHLOROPHYLL ML.$^{-1}$ (GRAM FRESH WT)$^{-1}$

DG	LG	LY
0.101	0.041	0.004

Table 1.—Amount of chlorophyll extracted from ten day old seedlings of each genotype.

converted to a per unit chlorophyll basis and are illustrated in Figure 3. The LY plants contain very little chlorophyll in their chloroplasts. The purpose of the second set of reactions was to determine the accuracy of the photoreductive curves obtained from suspensions containing very little chlorophyll. Here, the LG reaction mixture was increased from 4.0 ug of chlorophyll in the first reaction set to 30 ug of chlorophyll. The DG had an increase in chlorophyll content of 4.0 ug to 80 ug. A comparison of the time curves of the LG and DG containing small amounts of chlorophyll with the same containing large amounts of chlorophyll on a per unit chlorophyll basis shows them to be equal. A linear relationship between the amount of dye reduced exists with as small amounts of chlorophyll as 4.0 ug.

An increase of three fold in the photosynthetic reaction rate of the LG plastids over the DG plastids was found. A slight increase of LY over LG was also demonstrated. The ultrastructure of the LG and especially the LY mutant plastids exhibits a marked decrease in lipid content and lamellar stacking, Keck, Dilley, Allen and Biggs (1970). Since all of the data is expressed on a per chlorophyll basis, it is feasible that the higher rates of photoreduction exhibited by the LG and LY plastids are due to more chlorophyll proportionally involved in electron transport. However, Keck, Dilley and Ké (1970) attributed the higher reaction rates of LG chloroplasts to a greater plastoquinone pool and an altered lipid environment. Considering the more drastically altered lipid environment and expected greater plastoquinone pool of the homozygous mutant, one could predict the observed increase in photoreductive rates of the double recessive mutant. Since presently it is not possible to obtain plastids from each of the types used with comparable chlorophyll concentrations, the effects of disproportionate amounts of chlorophyll per reaction center must be considered.

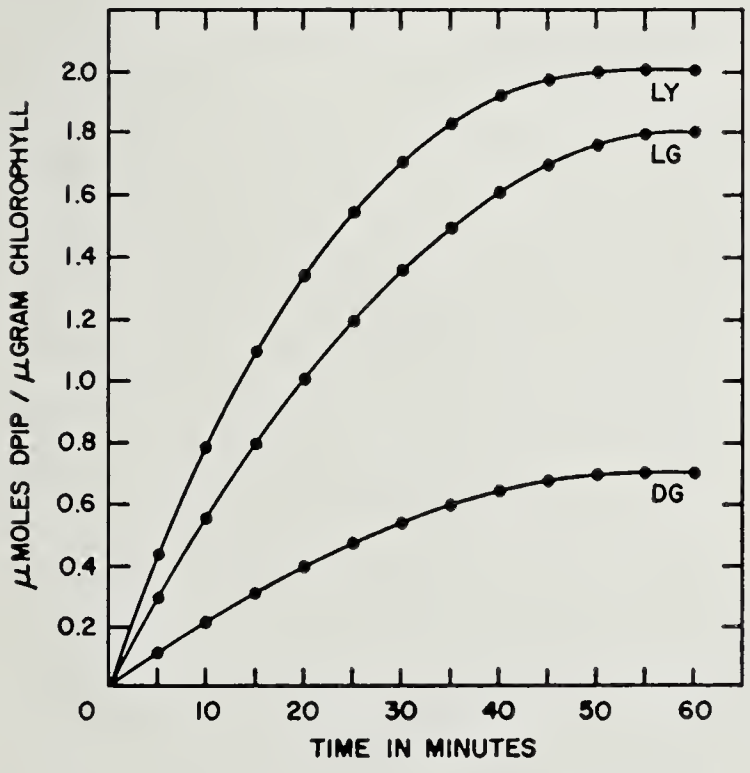

FIGURE 3.—Plot of umoles DPIP reduced per ug chlorophyll versus time. Electron transport of photosystems I and II using 4 ug chlorophyll from LY, 30 ug from LG, and 80 ug from DG.

ACKNOWLEDGMENTS

The authors wish to acknowledge Dr. Robert M. Chasson for his technical advice and for helping to prepare the manuscript. The authors also wish to express their appreciation to Dr. J. Louis Martens for his advice and consideration during the course of this study and for providing the seed stock used.

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Manuscript received November 22, 1971.

ALGAL STROMATOLITE DISTRIBUTION IN RELATION TO SUBSTRATE RELIEF: SHAKOPEE FORMATION (LOWER ORDOVICIAN), ILLINOIS

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ABSTRACT.—A quarry wall in the Shakopee Formation in north-central Illinois near Utica exposes a well-preserved algal stromatolite bed which averages 1.5 feet thick and extends for more than 1000 feet horizontally along the quarry walls. The contact between the algal bed and the underlying substrate shows considerable variation in topographic relief. Three distinct facies occur in the algal bed: continuous stromatolite layers, found in areas with little substrate relief; discontinuous stromatolites, which occur in areas of intermediate substrate relief; and a complex facies of mixed stromatolite morphologies, confined to an area of maximum relief. These associations suggest that substrate relief, which is an easily measurable parameter, may be more generally useful as a tool for paleoenvironmental interpretations of stromatolitic deposits in the geologic record.

Much research has been conducted on the effect of various physical environmental factors on the morphology and distribution of modern algal stromatolites (Neumann et al., 1970; Scoffin, 1970; Gebelein, 1969; Logan et al., 1964; Carozzi, 1962; Ginsburg et al., 1954; Black, 1933). Few studies, however, have dealt with the distribution of different stromatolite forms preserved in the geologic record (Hofmann, 1969).

Field studies of the Shakopee Formation in north-central Illinois revealed an excellent exposure of well-preserved stromatolites at the Utica Stone Quarry east of Utica. Distinct morphological forms of the stromatolites occur in a single horizon which extends laterally for over 1000 feet along the quarry walls. The contact with the underlying bed shows considerable variation in topographic relief and thus provides an opportunity to examine the effect of this substrate relief on the distribution of different stromatolite types.

GEOLOGIC SETTING

The Lower Ordovician Shakopee Formation overlies the New Richmond Sandstone and unconformably

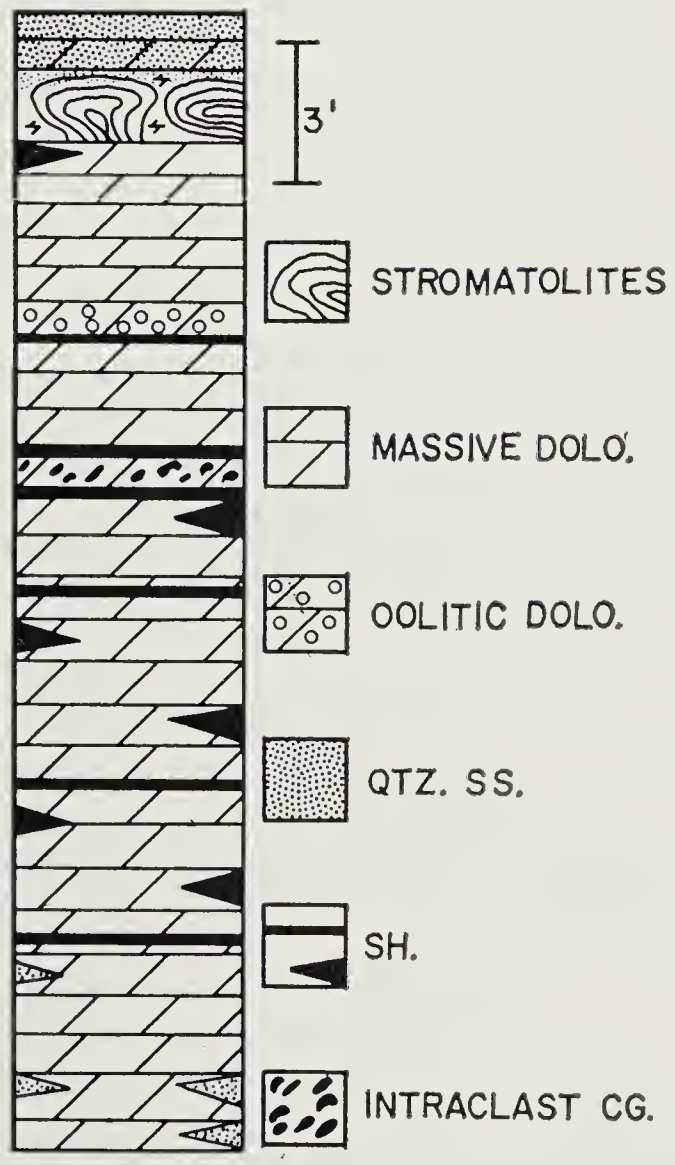


FIGURE 1.—Generalized stratigraphic section of the Lower Ordovician Shakopee Formation at the Utica Stone Quarry.

underlies the St. Peter Sandstone. The Shakopee deposits are composed of mainly underformed massive dolomites which contain sand and shale lenses, occasional intraclast conglomerates, a few continuous beds of calcareous shale and oolitic dolomite, mudcracks, cut-and-fill structures, cross-stratification and stromatolites, suggesting fluctuating shallow marine or intertidal conditions (Figure 1). The stromatolite horizon averages 1.5 feet thick and rests upon a substrate composed of continuous massive gray dolomite occasionally overlain by thin shale lenses. Parts of the section were obliterated through blasting.

A few cephalopods and gastropods are the only fossils other than stromatolites found at the quarry.

Facies Description

Since organic cellular structures are rarely preserved in stromatolites (Ginsburg et al., 1954) they are usually classified by geometrical characteristics (Hofmann, 1969; Logan ea al., 1964). According to most workers, the gross stromatolite morphology and lamination structures are the most important attributes in stromatolite classification. Changes in these attributes result from biological and physical variables (Hofmann, 1969; Logan et al., 1964). It has also been found that identical species of modern algae may generate different gross morphologies in different environments and, conversely, different algal species may exhibit similiar morphologies in the same environment.

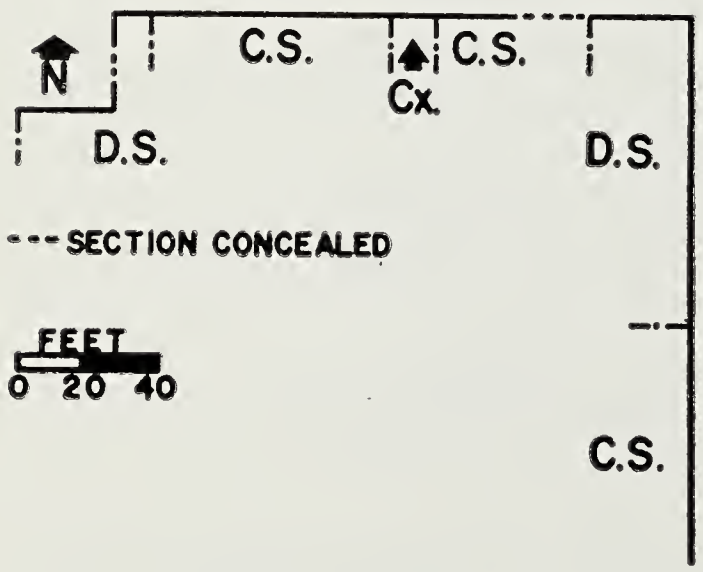


Figure 2.—Lateral distribution of stromatolite facies in a single horizon along the quarry walls. D.S.: Discontinuous Stromatolite Facies; C.S.: Continuous Stromatolite Facies; Cx.: Complex Facies.

Hofmann's (1969) classification was used to describe the laminations (which are composed of alternating grey and brown fine-grain dolomite) of stromatolite forms in this study. Three distinct facies are recognized (Figure 2): continuous stromatolite facies (C.S.), discontinuous stromatolites (D.S.), and a complex facies (Cx.).

Continuous Stromatolite Facies

The continuous stromatolites conform closely to substrate topography and extend uninterrupted for over 30 feet in length (Figures 2, 3). The main geometric arrangement of the laminations consists of close-linked undulating (stratiform) laminae (average thickness ¼ inch) and occasional small turbinate columns (relief up to 2 inches) which grade vertically into flat stratiform laminae (average thickness $\frac{1}{16}$ to ⅛ inch) with a few stromatolite nodules near the top of the algal bed (Figure 3). Locally discrete cylindrical columns (Figures 3, 4a) and biscuits (Figure 4b) occur in depressions. Columns have an average basal diameter of 2.5 inches and heights of approximately 4 inches. Biscuits have an average diameter of 11 inches and heights of up to 5 to 6 inches. These discrete biscuits and columns grade up into the undulating and flat stratiform laminae of the upper parts of the facies.

In this continuous facies (Figure 3) the substrate has very little relief. In transition zones which laterally con-

nect the continuous and discontinuous facies (Figure 3) the substrate consists of discrete mounds upon which small domes cap and then coalesce up section forming the typical continuous stromatolite lamination (Figure 3).

Discontincous Stromatolite Facies

The main distinction between the continuous and discontinuous stroma-

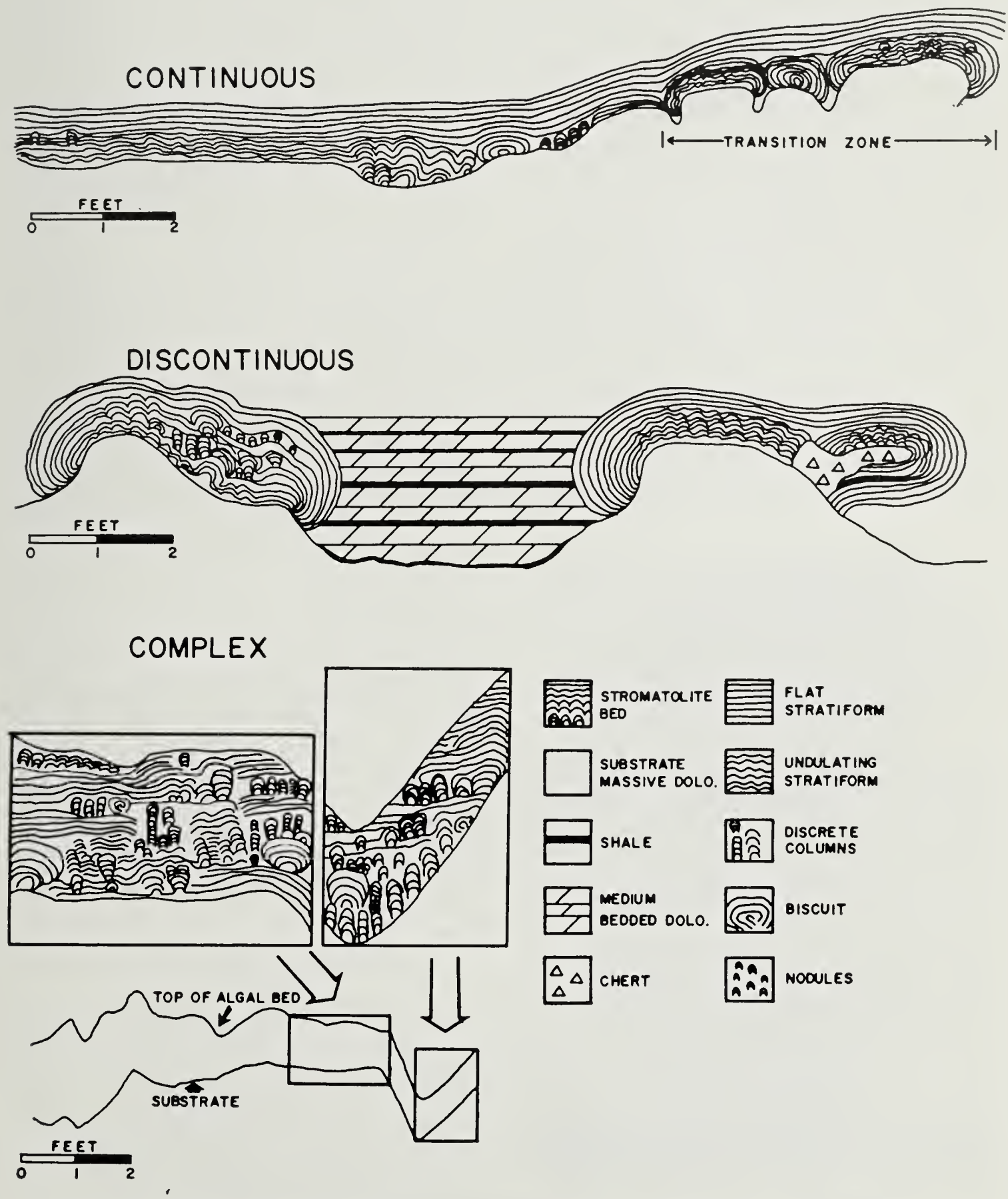


FIGURE 3.—Cross section of typical stromatolite sections exposed along the quarry walls.

tolite facies is the continuity of the algal bed. In the discontinuous stromatolite facies the algal structures cap substrate highs and vary between 1.5 and 7 feet in length. The discrete dome or head-shaped stromatolites are separated by low depressions where the substrate is more irregular (Figure 3). The interdome depressions are up to 3 feet lower than the adjacent stromatolite-capped highs and are filled with medium-bedded dolomite alternating with thin shale lenses. Depressions vary between 2 and 30 feet in width.

None of the individual laminations (average thickness $\frac{1}{16}$ inch) of the algal structures are truncated. Laminations gradually lap upon or wrap around substrate highs (Figure 3), indicating that these discontinuous structures are not merely erosional remnants of a previous continuous layer .Generally the internal structure of the laminations is the same as that found in the continuous facies, where discrete columns anastomose and grade up into undulating and flat statiform laminae.

Complex Facies

A complex facies of randomly associated biscuits, columns, anastomosed columns, undulating and flat stratiform and nodular laminae occurs in an area of maximum substrate relief in one place along the north wall (Figure 3). In contrast to the other facies, there is no definite vertical gradation from columnar and biscuit-shaped structures to flat-lying laminae near the top of the algal bed. Laminations are generally discontinuous and have variable thicknesses.

FIGURE 4.—Slab sections of typical small discrete cylinrical columns (a) and biscuits (b) of continuous stromatolite facies.

PROCEDURE

Smith's (1970) "bed relief index" (BRI) was modified to obtain quantitative estimate of the amount of substrate relief associated with the different stromatolite facies. Distances were measured from a leveled carpenter's cord base line which was laid out beneath the substrate bed. The BRI was calculated by subtracting the vertical distance measured from the base line of all relative lows from the vertical distance of each adjacent relative high and dividing this sum by the length of the base line (Figure 5). The resulting quotient is then multiplied by 10. Thus,

$$BRI = \frac{2[E_1+...+E_n)-(e_1+...+e_n) \pm Ex,y]x10}{L}$$

where $E_1+...+E_n$ represents elevations of relative highs, $e_1+...+e_n$ elevations of relative lows, and $E_{x,y}$ the elevations at the extreme ends of the traverse, the sign of which depends on whether they are low or high points. (Smith, 1970, p. 2998). In the above equation and Figure 5 the letter L represents the total length of the base line.

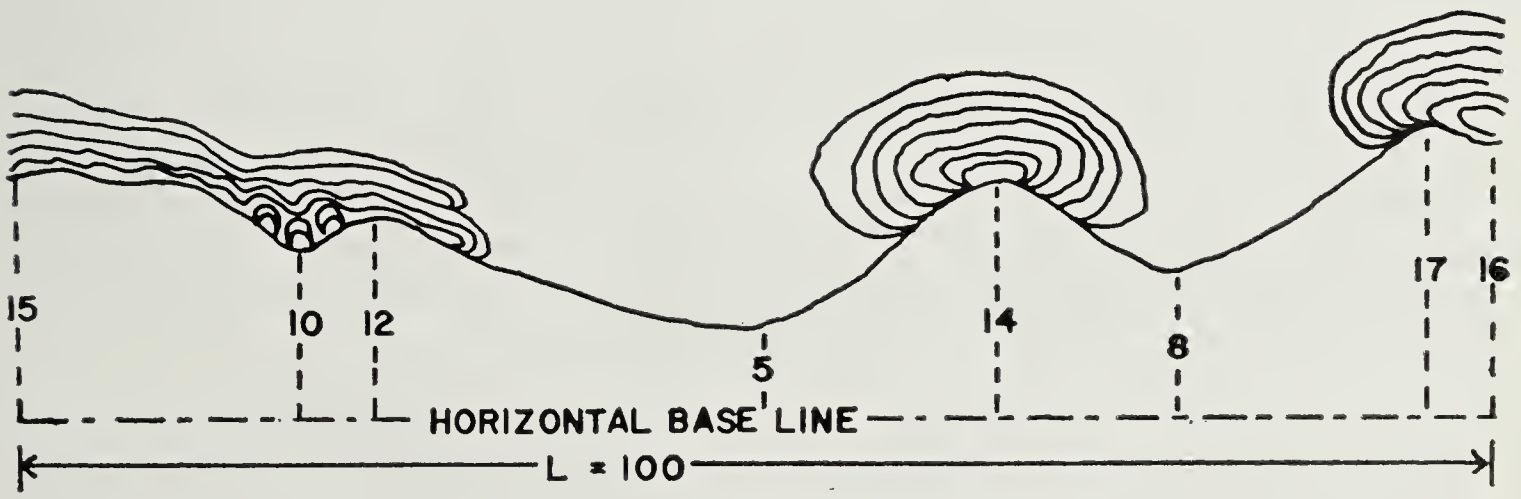
Bed relief indices were measured for the substrate under the different algal stromatolite facies. Horizontal traverses varying in length from 50 to 90 inches were measured at 26 representative sections of the algal bed. Because portions of the bed are naturally discontinuous and some regions were destroyed by blasting, traverse lengths were determined by the continuity of the exposure. The average traverse length was arbitrarily chosen to be 60 inches because of the many relief changes which occur over short horizontal distances.

Results

Field data indicate a correlation between the amount of substrate relief and the type of stromatolite facies. Flat continuous stromatolites are found in areas of low substrate relief along the north and east quarry walls (Figure 2) where the mean BRI is 2.07 with a standard deviation of .71. In both regions (Figure 2) the flat stromatolite layers lap off substrate highs and extend continuously for over 30 feet in shallow basins. Discontinuous forms occur in areas of greater substrate relief with an average BRI of 4.25 and standard deviation of .79. Here stromatolites cap substrate highs and do not occur in the depressions between algal domes. The complex facies with discontinuous laminations of variable thickness occurs in a local area along the north wall (Figure 2) with maximum substrate relief where the BRI is 7.02 and standard deviation of .75. A plot of stromatolite facies in relation to substrate bed relief index illustrates these relationships (Figure 6).

Discussion

Differences in location, exposure, tidal amplitude, substrate relief, rates of sediment movement and current



$$\text{SUBSTRATE BRI} = (15-10)+(12-10)+(12-5)+(14-5)+(14-8)+(17-8)+(17-16) \times 10$$

$$= \frac{2[(12+14+17)-(10+5+8)]+15-16}{100} \times 10$$

$$= 3.90$$

Figure 5.—Calculation of substrate bed relief index (BRI) modified after Smith (1970).

velocity produce morphologic differences in modern stromatolites (Gebelein, 1969; Ginsburg et al., 1954; Logan et al., 1964). Although it is rarely possible to say that any one environmental factor is responsible for a particular stromatolite form, some workers have correlated stromatolite morphologies with given sets of environmental conditions.

Gebelein (1969) found that stromatolite morphology, distribution and abundance are controlled by the energy of the environment, expressed in terms of current velocity and rates of sediment movement. Flat algal mats occur where surface current velocity is less than 15-20 cm/sec and bottom sediment movement is less than 60-80 gm/hr/ft. Discontinuous algal domes and biscuits form between 1 and 11 cm/sec current velocity and between 8 and 60 gm/hr/ft bottom sediment movement. Continuous mats form in either high or low energy environments and discontinuous structures form in intermediate energy environments.

Hoffman and others (in press) have also noted that the environmental setting is a main control of the morphology of stromatolites in Shark Bay, Australia. In low intertidal areas, flat smooth mats are found in protected embayments, low elongate domes are found along semi-protected coasts, and high circular bulbous-columnar stromatolites occur in exposed headlands. In intertidal zones the degree of wave turbulence and sediment movement control the gross morphology of stromatolites.

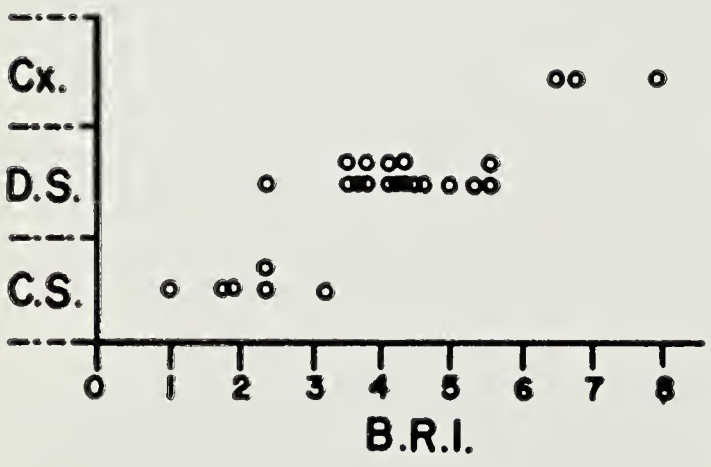


FIGURE 6.—Relationship of stromatolite facies and bed relief index (BRI) of the substrate. Cx.: Complex facies; D.S.: Discontinuous stromatolite facies; C.S.: Continuous stromatolite facies.

In light of these recent studies of modern stromatolites possible interpretations can be made for the Shakopee deposits. Flat continuous stromatolites which occur in areas of low substrate relief show no evidence of reworking or scouring of the algal bed. These continuous stromatolites may have been deposited in low energy basins or protected embayments. Discontinuous head or dome-shaped stromatolites with intermediate substrate relief may have occurred in higher energy zones where stromatolites grew on substrate highs, separated by small depressions which later became filled with sediment. The complex facies occurs in a local area with maximum substrate relief and contains discontinuous laminations of variable thickness. It may represent a tidal channel where current velocity and rates of sediment movement were highly variable.

Conclusions

Much work has been done to define the ecological range in which different modern stromatolite morphologies are found. Many of these studies have concentrated on environmental factors which are difficult to estimate in ancient stromatolitic deposits. The data of this study suggest that certain stromatolite associations correlate well with substrate relief, which along with other sedimentary structures may give a clue to the energy of the environment in which the stromatolites grew.

Acknowledgments

The author is grateful to N. D. Smith for many rewarding discussions through out the study. He, C. D. Gebelein and K. S. Rodolfo critically reviewed the manuscript and offered many helpful suggestions. Margaret M. Kinnally and S. Zyrkowski assisted in the field. The writer wishes to thank Mike Sitterly of the Utica Stone Quarry for his cooperation.

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Manuscript received July 6, 1971.

ADDITIONS TO THE FLORA OF ILLINOIS

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ABSTRACT.—*Arundo donax, Carex alata, Carex prasina, Populus nigra, Carya x lecontei, Stellaria pubera, Polycnemum majus, Eryngium prostratum,* and *Lactuca hirsuta* are reported from Illinois for the first time. Significant range extensions for a few other species are also noted.

The distribution records reported in this paper are the result of collections made during my field work for the Illinois Nature Preserves Commission as well as my own personal collections of plants from southeastern Illinois. In addition to the nine taxa reported from Illinois for the first time, range extensions are given for seven other species. A voucher specimen of each taxon reported has been deposited in the herbarium of Southern Illinois University (SIU).

NEW TO ILLINOIS

Arundo donax L. I observed a stand of this giant reed grass for five years before finding it in flower. During this time it continued to spread by rhizomes until it now forms a clone some 30 to 40 feet long along a county road embankment about two and one-half miles west of Mounds in Pulaski County. I collected it in flower on 24 Oct 1970, *Schwegman 2233a.*

Carex alata T. &. G. Previous reports of this sedge from Illinois were referred to *C. brevior* or *C. suberecta* by Jones and Fuller (1955). I collected this rather large sedge with crowded ovate spikes at the south edge of a narrow cypress swamp just south of Round Pond Swamp, one mile west of New Liberty, Pope County on 19 Jun 1971, *Schwegman 2271.* Although new for Illinois, this record represents only about a 60 mile extension of range eastward from a station reported in Scott County, Missouri by Steyermark (1963).

Carex prasina Wahlenb. The western limit of range of this sedge is given as Michigan by Fernald (1950). However, Hartley (1966) cites a specimen from low springy woods at Devil's Lake, Sauk County, Wisconsin. The collection reported here is from a spring-fed sedge meadow at Siloam Springs State Park in Adams County where I collected it 24 May 1971, *Schwegman 2269.* This locality represents a 250 mile range extension to the southwest. There are many springs in the park that appear to be suitable habitat for this species, but I found it in only one relatively small meadow near the Brown County line. The drooping spikes with a few perigynia in the terminal spike and the nerveless perigynia with spreading beaks are good field characters of the species.

Populus nigra L. Although the lombardy poplar (*P. nigra var. italica*) has been reported as spontaneous along the beaches of Lake Michigan and elsewhere in Illinois, this appears to be the first report of the typical variety growing wild in the state. I collected it along a gravel road in the southeast quarter of the southeast quarter of Section 8 about one mile southwest of Herod, Pope County on 8 May 1971, *Schwegman 2268.* I have observed it at this location for several years and it continues to spread in the old field habitat in which it occurs.

Carya x lecontei Little. Mr. Max Hutchison and I discovered two trees of this hybrid hickory at Ft. Massac State Park, Massac County on 20 Dec 1971, *Schwegman 2281.* It is a hybrid between *Carya aquatica* and *C. illinoensis.* The trees occur on the east side of a slough on the flood-

plain of Massac Creek near its mouth at the Ohio River. Two specimens of *C. aquatica* grow just north of the hybrids and a single large *C. illinonsis* stands just southeast of them. The nuts of this hybrid combine characters of both parent species.

Stellaria pubera Michx. The great chickweed has been previously collected in Illinois (Cook County) where it was discovered by Julian Steyermark. Swink (1969) notes that there are no other known localities for this eastern species in the Chicago region, and states that since there is evidence of planting by a wildflower fancier in the vicinity of Steyermark's collection it is uncertain as to whether the Cook County plants are native or introduced. The plants reported here occur along both forks of Burke Creek in the southwest quarter of Sect. 4, T 15 S, R 6 E in Pope County where I collected it 13 Apr 1968 *Schwegman 1540*. It is common in western Kentucky and is no doubt native at this Pope County locality.

Polycnemum majus A. Br. This member of the Chenopodiaceae has rarely been reported as an introduced weed in eastern North America. I collected it along the Missouri Pacific Railroad tracks about two miles southeast of Fults in Monroe County on 28 Jul 1970, *Schwegman s .n.* I found only a single specimen.

Eryngium prostratum Nutt. This southern species of moist soil has long been known to occur in Western Kentucky and southeastern Missouri, but this appears to be the first report of it from Illinois. I collected it from the east shore of Teal Pond at the U.S. Forest Service Teal Pond Campground four miles northwest of Eddyville, Pope County on 7 Aug 1971 *Schwegman 2272*. There were at least two stands of this low mat-forming species present at the time of my collection.

Lactuca hirsuta Muhl. I collected this wild lettuce from a roadside near Little Saline River two miles north of McCormick in Pope County on 16 Jul 1970, *Schwegman s. n.* It grows in dry open woods and occasionally on disturbed roadsides. This eastern species has not previously been reported adjacent to Illinois, however I have collected it in western Kentucky as well as in southeastern Illinois.

Range Extensions Within Illinois

Dryopteris cristata (L.) Gray. The crested wood fern is known to occur in northeastern Illinois, with La Salle County being its most southwesterly reported station. I collected it in peat soils of a large spring along the south side of Spring Lake in western Tazewell County on 8 Aug 1971, *Schwegman 2280.*

Commelina erecta L. This dayflower is generally distributed in sandy soil areas of northern and western Illinois but has not previously been reported from the southern tip of the state. I collected it 8 Aug 1970, *Schwegman s. n.* along a railroad near the Ohio River north of Golconda, Pope County. Since that time, I have observed it at numerous localities along the banks of the Ohio River from Shetlerville to New Liberty in Pope County.

Sisyrinchium atlanticum Bickn. I collected the slender blue-eyed grass in a remnant of a moist black-soil prairie along an abandoned railroad about three miles northeast of Gillespie, Macoupin County on 17 Jun 1971, *Schwegman 2270.* Its only other reported occurrence in Illinois is in Kankakee County. The Macoupin County area is about midway between the Kankakee County locality and stations reported for the species in southeastern Missouri.

Quercus prinus L. The rock chestnut oak has previously been reported

in Illinois only from the steep cherty slopes of the Ozark Plateau in Alexander County and western Union County. Mr. Larry Martoglio of the Shawnee National Forest called my attention to a stand of this tree on Murray Bluff in southern Saline County. I collected specimens there on 8 Feb 1970, *Schwegman 2229a.* The trees are adjacent to a blacktop road and probably represent the most accessible stand of this rare tree in Illinois. It occurs on a northwest-facing slope northeast of the "gap" in Murray Bluff in the southeast quarter of Sect. 26, T 10 S, R 5 E. Attempts to locate additional stands of this tree in southeastern Illinois have proved futile.

Thaspium barbinode (Michx.) Nutt. This plant was found growing on a cool, shaded sandstone ledge in a ravine adjacent to Lusk Creek west of the center of Sect. 11, T 12 S, R 6 E in Pope County. I collected it 4 Jul 1970, *Schwegman s. n.* In Illinois it has not previously been reported south of Washington County.

Liatris ligulistylis (Nels.) K. Schum. Wunderlin (1966) reported this species from Illinois for the first time on the basis of a Cook County specimen. The stand reported here was brought to my attention by Mr. Henry Eilers of Litchfield. It grows in a tall-grass prairie remnant south of the road and just east of the dam at Lake Lou Yaeger about four miles east of Litchfield in Montgomery County. I collected it 29 Sep 1970, *Schwegman s. n.* Steyermark (1963) reports this species from several counties bordering the Mississippi River in Missouri about 75 miles west of the population reported here.

Cacalia suaveolens L. This large conspicuous composite grows in wet floodplain woods and on the margins of springfed swamps along Little Saline River in northwestern Pope County about two miles north of McCormick. I collected it in flower on 5 Sep 1970, *Schwegman s. n.* It was previously reported no farther south in Illinois than Wabash County.

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Manuscript received January 7, 1972.

A BRIEF HISTORY OF LEPIDOPTEROLOGY IN ILLINOIS

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ABSTRACT.—Information is presented regarding the principal lepidopterists, past and present, who have worked in Illinois. The present location of their collections, if known, is given.

For several years, Dr. John C. Downey and the author have been collaborating in a distributional study of the butterflies of Illinois. During this project, much information concerning the history of butterfly collecting and study in Illinois has been obtained which, while too detailed for inclusion in a faunal check list, should be placed on record.

Mills (1958) and Ross (1958) present excellent accounts of the general history of biological research and faunistic surveys in the state. Their accounts do not make specific reference to butterflies, but several historical figures they discuss made important contributions to lepidopterology at an early period, when Illinois was still looked upon as the "West." A number of butterfly taxa were described from Illinois material during this early period; although some names are synonyms, many are still valid.

Botanical collecting took place in Illinois as early as 1795 (Jones and Fuller, 1955, p. 1), to which it is speculated that some entomological observations may well have been incidental. The earliest recorded butterfly observation and collecting was done by Edward Doubleday, an eminent English entomologist, who visited Illinois in the late autumn of 1837, during a two-year visit to the United States (1837-1838). He entered the state at Shawneetown, on the Ohio River, and made Salem, Carlyle and Belleville. From his way to St. Louis by way of Carmi, St. Louis he ascended the Mississippi River to Alton, thence up the Illinois River, passing Peoria, Peru and Ottawa, which he described. From the Illinois he reached Chicago, passing through Plainfield, and at Chicago he embarked on a lake vessel. His account of the trip (Doubleday, 1838) is delightfully written, but contains little entomological observation; it is mostly a commentary on the Illinois country, which was still in the stage of pioneer settlement at that early period. The insects which Doubleday collected during his journey are now in the British Museum.

The earliest resident worker to make significant contributions to the knowledge of the Illinois butterfly fauna was Benjamin Dann Walsh. The first official State Entomologist of Illinois, he began his studies in 1860 and continued then until his death nine years later. He was a correspondent of W. H. Edwards and supplied the latter with much Illinois material, from which Edwards described several butterfly taxa. These descriptions are among the earliest specific references to Illinois butterflies. The main portion of Walsh's collection, following his death, was deposited in the Chicago Academy of Sciences, where it was destroyed in the Chicago Fire of 1871. Some of it, however, had been retained by his successor as State Entomologist, William Le Baron, and became the nucleus of the present collection of the Illinois Natural History Survey. Although some of his material may even now be extant in that collection,

it is unfortunately unrecognizable, as many of the older specimens in the collection lack data.

Le Baron and Cyrus Thomas, the second and third State Entomologists of Illinois, published notes and incidental references to butterflies, chiefly from an economic point of view, in their official reports. Like those of Walsh, some of their specimens may still exist in the Natural History Survey collection, but cannot be identified.

G. M. Dodge lived and collected in Ohio Township, Bureau County, for some years before moving to Nebraska about 1874. His collecting furnished the basis for several records published by Edwards and Scudder, and he described *Hesperia illinois,* a synonym of *Euphyes bimacula* (Grote and Robinson), from Bureau County.

Charles E. Worthington of Chicago was a very active collector in northern Illinois in the 1870's and 1880's, but we have been unable to locate any biographical information about him. There is a letter in the Herman Strecker correspondence in the Field Museum of Natural History, which he wrote to Strecker on November 25, 1878, on the letterhead of J. B. Tascott and Sons, a paint manufacturer in Chicago; possibly Worthington was associated with this firm. He was the author of one of the two previously published lists of the butterflies of Illinois (Worthington, 1880). Like Walsh, he was a correspondent of W. H. Edwards, and provided material from which Edwards erected new butterfly taxa, among them *Cercyonis pegala olympus,* described from specimens reared from eggs sent to Edwards by Worthington from Chicago. Worthington himself proposed several new butterfly names, but all are synonyms with the exception of *Phocides pigmalion okeechobee.* We do not know the fate of his collection.

J. R. Muhleman collected in Macoupin County in the latter part of the 19th Century (Edwards, 1880: 113).

Thomas E. Bean lived at Galena, Jo Daviess County, in the late 1870's, and sent local material to Edwards, including a part of the type series of *Speyeria aphrodite alcestis.* Later he moved to Laggan (now Lake Louise), Alberta, continuing to supply material to Edwards from there.

George Hazen French served for many years on the faculty of Southern Illinois University at Carbondale, and did considerable local collecting. Some of his material is extant in the Field Museum of Natural History, the Illinois Natural History Survey, and the American Museum of Natural History, but none remains at the University. Although much of his taxonomic work was with the moths, he prepared the first published list of Illinois butterflies (French, 1879), in the form of a key to 113 species. An enlargement of this list formed the nucleus of his well-known popular manual "Butterflies of the Eastern United States" (French, 1885) which he produced while at Carbondale.

C. V. Riley lived in Illinois between 1860 and 1868, but appears to have done little if any butterfly work during that time.

W. E. Longley collected, mostly in and near Oak Park, at least between 1869 and 1905. A few Longley specimens are in the Field Museum, the Illinois Natural History Survey, the Carnegie Museum, and the United States National Museum.

Augustus H. Mundt collected at Fairbury, Livingston County, during the 1870's and 1880's. He corresponded and exchanged butterflies with Edwards, and published several short articles during that period. A biographical sketch was published by the present author (Irwin, 1966).

Selim Hobart Peabody taught high school physics in Chicago between 1865 and 1871, and from 1874 until 1878; in the interim, he served as professor of physics at the Massachusetts Argicultural College in Amherst. He came to the University of Illinois in 1878, and was Regent from 1880 until 1891. He too corresponded and exchanged butterflies with Edwards, and while in Chicago, wrote a popular book on insects for children (Peabody, 1869). His collection, including some of the butterflies he received from Edwards, is in the Natural History Survey, but is almost completely lacking in data.

A. J. Snyder, a high school teacher, collected during the 1890's, principally at Evanston. He published a local list (Snyder, 1896) and a revision of North American *Argynnis (Speyeria)* (Snyder, 1900). His collection was acquired by the Field Museum in 1904.

One of the largest and best-known collections of North American Lepidoptera was that of Dr. William Barnes of Decatur. At various times such prominent figures in American lepidopterology as Drs. James H. McDunnough, A. W. Lindsey and Foster H. Benjamin acted as curators of the collection. The emphasis of Dr. Barnes and his curators was on the butterflies of other parts of the United States, and largely on the moths. The collection was acquired by the United States National Museum in 1931.

Judge Richard M. Barnes, not related to the foregoing, collected in Marshall, Putnam and Woodford counties from the early 1920's until about 1944. It appears that he had other individuals, not themselves lepidopterists, collecting for him in this area at the same time. Irwin (1966) gave an account of his life and the disposition of his collection, the largest part of which is in the Illinois State Museum, with most of the remainder in the Illinois Natural History Survey.

Charles Robertson of Carlinville, a hymenopterist, made an insect collection from that area during the latter 19th Century. This collection, including many butterflies, is now in the Illinois Natural History Survey.

The collection of Andreas Bolter, principally of Lepidoptera and Coleoptera, is also in the Survey. It contains some interesting state records, but like many of his contemporaries, Bolter placed inadequate data on his specimens.

W. A. Nason of Algonquin also assembled a general collection of the insects of that area, which is in the Natural History Survey. His data are generally adequate.

The acknowledged dean of Illinois lepidopterists for many years was Alex K. Wyatt (Alexander Kwiat) of Chicago. He commenced his collecting activities as a young man about 1896 and continued them until the late 1950's. Wyatt's contributions to the study of Lepidoptera are extensive and well known. He served for a number of years as research associate at the Field Museum. He specialized in the Noctuidae, but his butterfly collecting, mostly in the Chicago area, yielded many rarities for the state. He turned over his fine collection to the Field Museum in 1957, incorporating it himself into the general study collection. Wyatt died May 14, 1971, at the age of 92.

A. G. Lauck collected near Alton for a number of years. His collection was acquired by Colin W. Wyatt of Surrey, England.

Oliver S. Westcott was a high school teacher in Chicago, and served for many years as principal of what is now Waller High School. He form-

ed a large insect collection, which was deposited in the State College of Washington at Pullman (Anonymous, 1920). An elementary school in Chicago now bears his name.

John L. Healy of Chicago was an organizer of the old Chicago Entomological Society and was its president at the time of his death in 1926. He had placed his collection in the Chicago Academy of Sciences shortly before his death (Wyatt, 1926).

Other collectors active in Illinois early in the present century, mostly in the Chicago area, included E. F. Lustig, A. Sala, A. Herz and A. L. McElhose, whose collections are in the Field Museum, and Emil Beer, whose material has been deposited in the Illinois Natural History Survey. The collection of Adolph Mares was divided between the two institutions; his North American butterflies went to the Natural History Survey and the exotics to the Field Museum. W. J. Gerhard, for many years curator of insects at the Field Museum, and Emil Lilieblad, assistant curator of insects there, collected Lepidoptera in the Chicago area and elsewhere in Illinois, although both were specialists in other orders. Early collectors about whom relatively little is presently known were E. B. Chope, C. Krueger and J. Tough; some of their specimens are in the Field Museum.

Leonard S. Phillips of Wheaton was an active collector in many parts of Illinois until his untimely death in 1968. Most of his collection was presented to Buena Vista College, in Storm Lake, Iowa; but a portion of it is still in the hands of Mrs. Phillips at the present time.

Dr. Charles L. Remington of Yale University collected and studied the butterflies of Elsah, Jersey County, from 1939 until 1943 (Remington, 1943).

Among presently active Illinois workers, Murray O. Glenn of Henry is prominent. He has collected in that area since 1913. Most of his research involves the microlepidoptera, of which he has a superb collection, but he also possesses a good local reference collection of butterflies.

Maurice L. Bristol of Elgin has an excellent collection; he has turned up a number of very interesting records in that area, including most of the known Illinois specimens of *Calephelis muticum* McAlpine.

Dr. John C. Downey was on the faculty of Southern Illinois University at Carbondale from 1956 until 1968, and he and his students collected intensively in southern Illinois. Their collections have been deposited at the University, and are the foundation of our knowledge of the present-day butterfly fauna of the lower third of the state.

The present author has collected in Illinois since 1941, principally in La Salle and Livingston counties near Streator and in the Chicago area, but has made collecting trips to all parts of the state. He has specialized in the study of Illinois butterfly distribution. Nearly all of his collection was turned over to the Natural History Survey in 1970.

Ogden T. Plumb of Streator collected in that area between 1942 and 1956, in collaboration with this author. His collection was acquired by the author and is now in the Natural History Survey.

Gary McCoy, who collected in the Streator area between 1962 and 1968, contributed many valuable records to the Illinois butterfly distributional study. Some of his material was obtained by the author and is now in the Natural History Survey, but most of it remains in McCoy's possession.

Theodore Ellis, Jr. of Danville has collected in that area for many years;

his records include the most recent Illinois capture of *Speyeria diana* (Cramer).

Other active collectors in Illinois include Richard A. Arnold of Hinsdale; Jackson Boughner of Chicago; Thomas G. Brady of La Grange Park; Patrick J. Conway of Chicago (Illinois material largely from Mercer County); Gregory Nielsen of Chicago; V. G. Sasko of Chicago (very large worldwide collection); Norman G. Seaborg of Lockport; and Thomas Taylor of Peoria (excellent representation from Peoria and Mason counties).

The author is aware that the above is not a complete account of all who have collected and studied Illinois Lepidoptera. Doubtless some collectors of an earlier day have been overlooked, while of present-day collectors and members of the Lepidopterists' Society who have not been mentioned, some he has not had the privilege of knowing personally and does not know what form their studies of the Lepidoptera have taken.

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Manuscript received February 7, 1972.

THE INCORPORATION OF RADIOACTIVE SUBSTRATES INTO PRODIGIOSIN BY *SERRATIA MARCESCENS*, STRAIN 75.

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ABSTRACT.—It is suggested from this research that the amino acids L-proline and L-histidine are utilized by *S. marcescens* for the production of prodigiosin. Gluconate is also incorporated into the pigment probably as ring compounds that are precursors for prodigiosin, whereas acetate plays a somewhat minor role in prodigiosin syntheses. Sucrose and L-tryptophan were found not to enhance early pigment production in these microorganisms.

The bacterium *Serratia marcescens* has received a great deal of attention primarily because of an orange-red pigment it produces called prodigiosin. Prodigiosin appears to be the only naturally occurring pyrryldipyrrylmethene (Rappoport and Holden, 1962).

Originally the prodigiosin molecule was thought to be a tripyrrylmethene (Wrede and Rothaas, 1934), but was later proposed to be pyrryldipyrrylmethene (Wasserman, 1960a). However, it is probable that prodigiosin is a combination of several closely related structures. Four distinct fractions had been separated from the pigment by Williams, Green, and Rappoport (1956a), and Weise (1949) using paper chromatography. More recently, six distinct fractions have been separated (Lynch, Worthy, and Kresheck, 1968).

The knowledge of the biosynthesis of prodigiosin and of its fractions is incomplete. Originally the succinate-glycine cycle was considered the pathway for prodigiosin production, (Hubbard and Rimington, 1950), because ^{14}c-glycine and ^{14}c-acetate were found to be incorporated into the pigment. Lately there has been evidence that the synthesis of prodigiosin does not follow the usual porphyrin-pyrrole scheme (Marks and Bogorad, 1960; and Shrimpton, Marks, and Bogorad, 1963). In addition, Shrimpton, et al, (1963) found that certain ^{14}C labeled amino acids (L-proline, L-ornithine, and glycine) were incorporated into the prodigiosin pigment.

The purpose of this investigation is twofold: ((1) To determine whether the amino acids, L-tryptophan, L-histidine, and L-proline, are incorporated into the pigment; and (2) to determine if the carbohydrates, sucrose, gluconate, and acetate, are utilized in pigment production.

The incorporation of these potential precursors into prodigiosin was monitored by measuring the uptake of radioactive substrates into the pigment molecules.

METHODS AND MATERIALS

Preparation of Growth Media

Serratia marcescens strain 75 was grown in 250 ml portions using the following mineral salts growth media developed by Bondarenko (1965).

K_2HPO_4	0.50g
KH_2PO_4	0.75g
$NaNO_3$	0.75g
$(NH_4)_2SO_4$	0.50g
NaCl	0.15g
glucose	2.50g
distilled H_2O	250.ml

Experiments with Amino Acids

The first phase of the investigation dealt with the determination of which amino acid and carbohydrate would adequately support pigmentation.

This was accomplished by preparing duplicate portions of growth media which were readied for inoculation as follows.

Flasks 1 + 2 Mineral salts growth media + 40 mg L-proline

Flasks 3 + 4 Mineral salts growth media + 40 mg L-tryptophan

Flasks 5 + 6 Mineral salts growth media + 40 mg L-histidine

Flasks 7 + 8 Mineral salts growth media only

Flasks 9+ 10 Mineral salts growth media + 40 mg acetate

Flasks 11 + 12 Mineral salts growth media + 40 mg sucrose

Flasks 13 + 14 Mineral salts growth media + 40 mg gluconate

Flasks 15 + 16 Mineral salts growth media only

Following these additions, the flasks were sterilized before inoculation.

The inoculum was prepared from *Serratia* cultures grown on nutrient agar slants, and the inoculum was harvested after 24 hours incubation by adding 10 ml of sterile distilled H_2O to the slants. The suspended cells were introduced aseptically into each of the sterile culture flasks.

The flasks were placed on a shaker and incubated for 24 hours at room temperature. Visual observations of pigment production and coloration were recorded (see Table I).

Investigations With Radioactive Pigment-Supporting Amino Acids

The next phase of the experiment dealt with the amount of amino acid and carbohydrates incorporated into the pigment. This was accomplished by preparing duplicate 250 ml aliquots of mineral salts media which were readied for growth as follows.

Flasks 1 + 2 Mineral salt growth media + 40 mg L-histidine + 1 uCi ^{14}C-L-histidine

Flasks 3 + 4 Mineral salts growth media + 40 mg L-proline + 1 uCi ^{14}C-L-proline

Flasks 5 + 6 Mineral salts growth media + 40 mg gluconate + 1 uCi ^{14}C-gluconate

Flasks 7 + 8 Mineral salts growth media + 40 mg acetate + 1 uCi ^{14}C-acetate

Following sterilization and inoculation, the culture flasks were placed on a shaker and incubated for 72 hours at room temperature. The cells were harvested from the suspension by centrifugation in an IEC B-20 centrifuga at 12,000 x g for 15 minutes.

Extraction of Pigment

The supernatants of all the replicates were decanted and saved for further analyses. Using a procedure developed by Lynch in this laboratory for pigment extraction, the sedimented cells from each flask were treated with 25-50 ml of glacial acetic acid and

TABLE *I.*—Effect of L-proline, L-tryptophan, and L-histidine on coloration and pigment production after 24 hours incubation.

Flask	Amino Acid	Coloration	Pigment Production
1	L-proline	Deep pink	Very heavy
2	L-proline	Deep pink	Very heavy
3	L-tryptophan	Faint pink	Very light
4	L-tryptophan	Faint pink	Very light
5	L-histidine	Salmon	Very heavy
6	L-histidine	Salmon	Very heavy
7	None	Faint pink	Very light
8	None	Faint pink	Very light

were then placed on a shaker for several hours. This solution of acid and cells was then centrifuged again at 12,000 x g for 15 minutes. The supernatants (which contained the pigment) were decanted and stored in the refrigerator for further analyses. The sedimented cells were digested with 2-3 ml concentrated KOH for several hours.

Preparation of Replicates for the Scintillation Counter

The portions left of media and the pigment extractions were labeled media (the first supernatant), pigment (the second supernatant), and cells (the cell debris after digestion). There were two replicates of cells, pigment, and media for each amino acid. Each replicate was prepared for counting in the following manner:

(1) 0.1 ml of each replicate was placed in separate counting vials.

(2) 5 ml of ethanolamine and 10 ml of fluor solution was added to each of the vials.

(3) a control was prepared by adding 5 ml of ethanolamine and 10 ml of fluor to a counting vial.

(4) 0.1 uCi of each labeled amino acid was placed in separate vials along with 5 ml ethanolamine and 10 ml of fluor solution to obtain total counts for each labeled substrate.

The vials were counted in a Nuclear Chicago Unilux II scintillation counter for 10 minutes. Average values were calculated and recorded in Table III.

Information on Radioactive Materials

All the radioactive materials were obtained from New England Nuclear Inc. in 50 uCi samples. The amino acids were uniformly labeled. The acetate was labeled in the carboxyl carbon and the gluconate in the number 6 carbon.

Results and Discussion

Results of Investigations With Amino Acids

Initial results revealed that of the three amino acids tested, L-proline, and L-histidine enhanced pigment synthesis in *Serratia* whereas L-tryptophan yielded only a small amount of pigment (Table I) even after 72 hours of incubation.

Since this strain of *Serratia* produces pigment very slowly in a glucose salts media unsupplemented with amino acids, the extensive early pigmentation can be attributed to the addition of the amino acid substrates.

With L-proline, an early, intense pink pigment occurred in all culture flasks of *S. marcescens*. Shrimpton, et al, (1963) also found L-proline to be intimately involved in pigment formation.

Since L-tryptophan failed to stimulate pigment production further in-

Table II.—Effects of acetate, sucrose, and gluconate on pigment coloration and production in *Serratia marcescens*, strain 75, after 24 hours incubation.

Flask	Carbohydrate	Coloration	Pigment Production
1	Acetate	Salmon	Heavy
2	Acetate	Salmon	Heavy
3	Sucrose	Faint pink	Slight
4	Sucrose	Faint pink	Slight
5	Gluconate	Deep pink	Heavy
6	Gluconate	Deep pink	Heavy
7	Glucose only	Faint pink	Slight
8	Glucose only	Faint pink	Slight

vestigations with L-tryptophan were discontinued.

The amino acid L-histidine exhibited some involvement in pigment production and growth since the flasks containing L-histidine produced a heavy crop of cells and an abundance of salmon-colored pigment. Since no published data exist concerning the incorporation of L-histidine into prodigiosin, the heavy incidence of pigment suggested that radioactive studies with this substrate should be pursued.

Results of Investigations With Carbohydrates

Tentative results using carbohydrates indicate that acetate and gluconate support some pigmentation whereas sucrose does not support pigmentation in this strain of *Serratia* (see Table II).

The mineral salts media plus acetate allowed the *Serratia* to grow abundantly and to produce extensive pigmentation. These data with acetate are consistent with earlier findings of Hubbard and Rimington (1950) in their prodigiosin studies. A literature search showed that no published data concerning the incorporation of gluconate into prodigiosin are available. Hence, this substrate was selected for further studies.

Results of Investigations With Radioactive Proline

One microcurie of uniformly labeled L-proline-^{14}C was added to each flask of growth media and incubated after inoculation for 72 hours. An intense pink pigment was formed. After the pigment was extracted, it was examined in the scintillation counter. Of the recovered amino acid L-proline, 36.66 per cent was found in the pigment. Of the introduced 1 uc of radioactivity, 44.84 per cent was recovered in the pigment, cell, and media replicates (see Table III). Shrimpton, et al., (1963) had found a 36 per cent incorporation of L-proline into the pigment which is entirely consistent with the data reported here. Since the amino acid used in this research is uniformly labeled, it is impossible to determine exactly what portion of the L-proline molecule is incorporated into the pigment. It seems consistent to suppose that proline donates its entire ring structure to the prodigiosin molecule, i.e., a 2-methyl-3-amyl pyrrole group. There is a striking similarity in structure between L-proline and 2-methyl-3-amyl pyrrole.

Results of Investigations with Radioactive Histidine

When radioactive histidine was added to the growth media, abundant pigmentation was observed in the culture flasks. About 36.29 per cent of the ^{14}C from uniformly labeled L-

TABLE III.—Values for uniformly labeled L-histidine-^{14}C and L-proline-^{14}C in the replicates of cells, media, and pigment; also shown are the per cents of recovery and incorporation of the labeled amino acids.

Amino acid	Vial number	Media (counts per 10 minutes)	Pigment	Cells	Per Cent incorporation	Per Cent recovery
L-proline-^{14}C	1	642,000	450,600	58,050	36.66	44.84
L-proline-^{14}C	2	660,000	451,200	197,850		
L-histidine-^{14}C	1	720,000	596,400	364,750	36.29	41.67
L-histidine-^{14}C	2	660,000	584,400	327,900		

histidine was present in the pigment with an overall recovery of 41.67 per cent (see Table III).

It seems probable that L-histidine would be incorporated into the *Serratia* pigment fraction under natural conditions. Since the L-histidine-^{14}C was uniformly labeled, it is impossible to determine exactly what portion of the L-histidine molecule was incorporated. However, L-histidine has an imidazole ring which differs substantially from the pyrrolic structures found in prodigiosin. It is probable that the imidazole group of L-histidine cannot be utilized directly in the pigment molecule. Consequently it is likely that either a portion of the imidazole ring or a side chain of the L-histidine molecule is utilized for pigment formation. The exact role of L-histidine in pigment synthesis thus awaits studies with variously labeled L-histidines, but since 36.29 per cent of the recovered radioactivity from L-histidine was present in the prodigiosin molecule, its role in pigment biosynthesis is not a minor one.

Results of Investigations With Radioactive Gluconate

After 72 hours of incubation, *Serratia* had produced the characteristic wine-colored pigment in mineral salts media amended with gluconate-^{14}C. Following its extraction and examination in the scintillation counter, about 32.09 per cent of the recovered gluconate-^{14}C was incorporated into the pigment. Approximately 28.07 per cent of the initial radioactivity was recovered in the pigment, cells, and media fractions (see Table IV). The remaining radioactivity was respired as $^{14}CO_2$.

Since only the sixth carbon atom of the gluconate was labeled, it probably was incorporated into the pigment fraction as a side chain grouping. Gluconate is a six carbon unsaturated chain from which one or more carbons could easily be removed. Another possibility although less likely, is that the gluconate carbon chain is rearranged into a ring structure from which a pyrrole might be formed. Also, if gluconate is utilized by way of the pentose cycle, erythrose-4-phosphate is produced which may condense with phosphoenolpyruvate to form 2-keto-3-deoxy-7-heptonic acid, a key intermediate in the biosynthesis of aromatic amino acids.

Results of Investigations With Radioactive Acetate

When radioactive acetate was used as the carbon source in the *Serratia* culture flasks, 3.64 per cent of the recovered, labeled substrate was present in the pigment. Approximately 79.94 per cent of the total initial acetate-^{14}C was recovered (see Table IV).

The acetate-^{14}C was labeled in the carboxyl carbon only. Since only about 3.64 per cent of the ^{14}C-acetate was incorporated into the pigment,

TABLE IV.—Values for gluconate-^{14}C and acetate-^{14}C in the replicates of cells, media, and pigment; also shown are the per cents of recovery and incorporation of the labeled carbohydrates.

Carbohydrate	Vial number	Media (counts per 10 minutes)	Pigment (counts per 10 minutes)	Cells (counts per 10 minutes)	Per Cent incorporation	Per Cent recovery
Acetate-^{14}C	1	5,742,000	238,200	54,800	3.64	79.94
Acetate-^{14}C	2	6,135,000	215,400	48,000		
Gluconate-^{14}C	1	1,407,000	644,400	205,000	32.09	28.07
Gluconate-^{14}C	2	1,146,000	794,400	286,400		

only a modest proportion of the COOH groupings in acetate are used for pigment biosynthesis.

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Manuscript received February 7, 1972.

STUDIES ON THE PHENOL-SOLUBLE LIPOPOLYSACCHARIDES FROM *SERRATIA MARCESCENS* BIZIO II. IMMUNOCHEMICAL AND ELECTROPHORETIC CHARACTERIZATION

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ABSTRACT.—The phenol-soluble lipopolysaccharide (fraction LPS-GP-I) and the lipoglycoprotein (fraction LPS-GP-II) isolated from endotoxin of *Serratia marcescens* Bizio were characterized by polyacrylamide gel electrophoresis, immunodiffusion and immunoelectrophoresis. By these techniques, it is concluded that fraction LPS-GP-II is homogeneous while fraction LPS-GP-I is heterogeneous. Fraction LPS-GP-I was found to be immunogenic and possesses a common antigenic determinant with fraction LPS-GP-II, the water-soluble lipopolysaccharide (fraction LPS-AB) and the sodium dodecyl sulfate extracted cell wall glycoprotein. Degradative procedures are proposed for further characterization of the phenol-soluble and the water-soluble lipopolysaccharides.

It is generally accepted that when endotoxin (lipopolysaccharide-protein complex) or whole cell of Gram-negative bacteria is submitted to 45% hot aqueous phenol extraction (Westphal, 1952), the aqueous phase contains the lipopolysaccharide fraction while the phenol phase contains the "simple protein" of the endotoxin (Wober and Alaupovic, 1971). However, several phenol-soluble lipopolysaccharides have been found to occur in several Gram-negative bacteria (Raff and Wheat, 1968). In a previous communication (Tsang, 1971), the isolation and chemical characterization of two phenol-soluble lipopolysaccharide-like materials from the endotoxin of *Serratia marcescens* Bizio was described. The major fraction (fraction LPS-GP-I) had the essential characteristics of a lipopolysaccharide, while the minor fraction (fraction LPS-GP-II), though containing the core components such as 2-keto-3-deoxy-octonic acid and heptose, had the composition of a lipoglycoprotein (14% fatty acids, 33% total carbohydrate, and 60% protein). This report represents the study of the immunochemical specificity and electrophoretic behavior of these two fractions.

MATERIALS AND METHODS

Serratia marcescens Bizio, grown on an inorganic medium, was supplied by General Biochemicals, Chagrin Falls, Ohio. Water-soluble lipopolysaccharide fractions (fraction LPS-WPB and fraction LPS-AB) were obtained from the aqueous phases of the hot aqueous phenol extraction (Westphal, 1952) of either the whole cells (fraction LPS-WPB) or endotoxin prepared by the modified Boivin method (Tsang and Rilett, 1970) (fraction LPS-AB). Phenol-soluble lipopolysaccharide-like materials (fractions LPS-GP-I and LPS-GP-II) were isolated from the phenol phases after the endotoxin was treated with hot aqueous phenol (Tsang, 1971). Sodium dodecyl sulfate extractable cell wall glycoprotein (fraction SBC) was prepared by the method as previously described (Tsang, *et al.*, 1971).

Polyacrylamide gel electrophoresis was performed on 7.5% polyacrylamide in 8 M urea, pH 9.5 according to the procedure of Davies (Davies, 1964). Electrophoresis was carried out in a Shandon Apparatus for 15

minutes at 5 mA in 0.05M Tris- glycine buffer, pH 8.3 at room temperature. The polyacrylamide gel columns were fixed in 20% sulfosalicylic acid for protein staining with 0.25% coomassie blue in water. All gel columns were destained by washing with many changes of 7% acetic acid.

Immunodiffusion was carried out according to the double diffusion technique in agar gel (Ouchterlony, 1962). For immunoelectrophoresis a solution of 1% agar in 0.5 M Veronal buffer, pH 8.6 was used as the supporting medium. Electrophoresis was carried out in a Gelman Delux Electrophoresis Chamber for 45 minutes at 10 mA per agar covered rack. Immunodiffusion was carried at 37 C° as previously described (Tsang, *et al.*, 1971).

Antisera were prepared according to procedures described elsewhere (Tsang, *et al.*, 1971). Briefly, antiserum against fraction LPS-GP-I was prepared by injection of white New Zealand male rabbits (4 to 5 lbs.) with saline solution (1 mg/dose). A total of 5 mg was administered in four sample injections at intervals of 5 days. The last injection consisted of a dose of 2 mg. The animal was bled by cardiac puncture at the end of 20 days. The anti-whole cell serum of *S. marcescens* Bizio was a gift from Prof. Petar Alaupovic of Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma. The presence of the antibodies in the sera was tested by double diffusion in agar gel.

Results and Discussion

The endotoxin or Somatic O-antigen complexes occurring in the outer layer of the cell walls of Gram-negative bacteria are macromolecular entities composed of lipids, polysaccharides, and proteins. In the general application of the 45% hot aqueous phenol fractionation procedure of the endotoxin or the whole cells, lipopolysaccharide (LPS) are isolated from the aqueous soluble phase and the phenol fractions are usually discarded. Recently, new attention has been paid to the phenol fraction because of the occurrence of several phenol-soluble LPS and the revived interest in the structure and functions of the protein moieties of the endotoxin which are soluble in the phenol phases as "simple proteins" (Wober and Alaupovic, 1971). In the previous studies of endotoxins of *S. marcescens* Bizio we reported the isolation of both water-soluble LPS (Tsang and Rilett, 1970), and the phenol-soluble LPS (Tsang, 1971). These two classes of LPS differed from one another mainly in that the latter contained twice as much fatty acids as did the former and was much less soluble in water. The similarity of composition and the wide range of solubility of LPS may suggest that the occurance of LPS in a particular solvent may be primarily a function of simple distribution. In order to support this suggestion, the present investigation was undertaken in an effort to describe more fully some of the other properties of LPS, namely immunochemical specificities and electrophoretic behaviour. The presence of any common antigenic determinants as well as similarity in electrophoretic mobilities of these two groups of LPS may reveal the common nature of these LPS fractions.

As reported in the preceding article (Tsang, 1971), the phenol-soluble materials isolated by ethanol precipitation could be resolved into two fractions (fraction LPS-GP-I and fraction LPS-GP-II). On polyacrylamide gel electrophoresis in 7.5% polyacrylamide gel employing a continuous buffer system (Tris-glycine, pH 8.6), the minor fraction (fraction LPS-GP-II) migrated as a single diffused

band (Figure 1, pattern B) while the major fraction (fraction LPS-GP-I) contained two diffused bands. The fast-moving component of fraction LPS-GP-I had the same mobility as the minor fraction (fraction LPS-GP-II). The identity of the fast-moving component of fraction LPS-GP-I and fraction LPS-GP-II was further illustrated by immunoelectrophoresis (Figure 2, pattern 1). Fraction LPS-GP-II, one of the components of LPS-GP-I and the water soluble LPS (fraction LPS-AB) shared similar mobilities and moved towards the cathode. Similar results were obtained for fractions LPS-GP-I and LPS-AB when antibody against whole cell was substituted with antibodies against fraction LPS-GP-I (Figure 2, pattern 3).

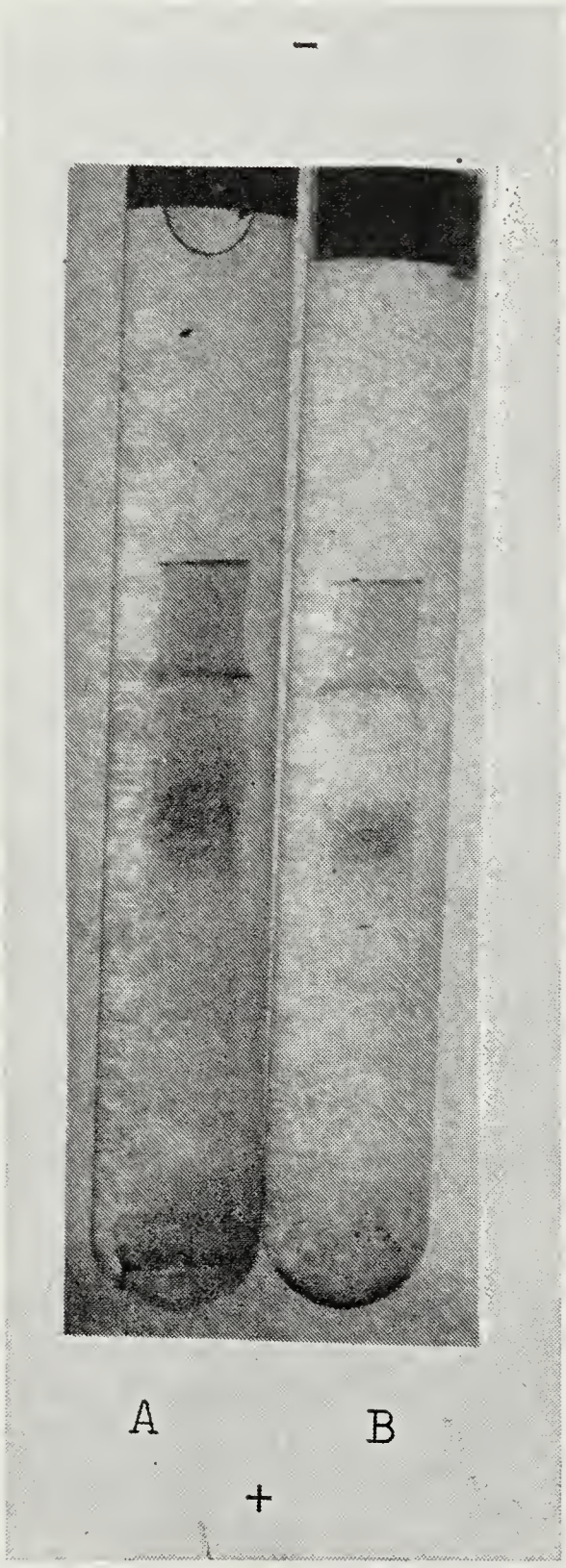

FIGURE 1.—Polyacrylamide Gel Electrophoresis of Fractions LPS-GP-I and LPS-GP-II.

A: Fraction LPS-GP-I
B: Fraction LPS-GP-II

From Figure 3, it can be seen that fraction LPS-GP-I formed two precipitin lines (pattern a) on immunodiffusion with antibodies against whole cell of *S. marcescens* Bizio but formed single precipitin lines with antibodies against either the sodium dodecyl sulfate extractable cell glycoprotein (fraction SBC) (pattern b) or with its own antibodies (pattern c). One of the two precipitin lines exhibited by fraction LPS-GP-I against anti-whole cell serum was in complete

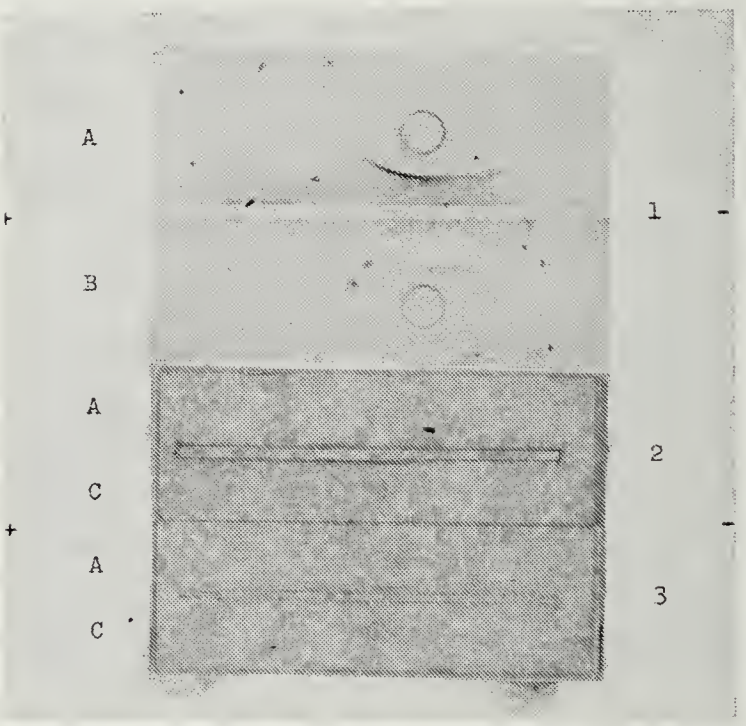

FIGURE 2.—Immunoelectrophoresis Patterns of Fractions LPS-GP-I, LPS-GP-II, and LPS-AB.

Central troughs in pattern 1 and 2 contain antibodies to whole cell of *S. marcescens* Bizio; central trough in pattern 3 contains antibodies to fraction LPS-GP-I.

A: Fraction LPS-GP-I
B: Fraction LPS-GP-II
C: Fraction LPS-AB

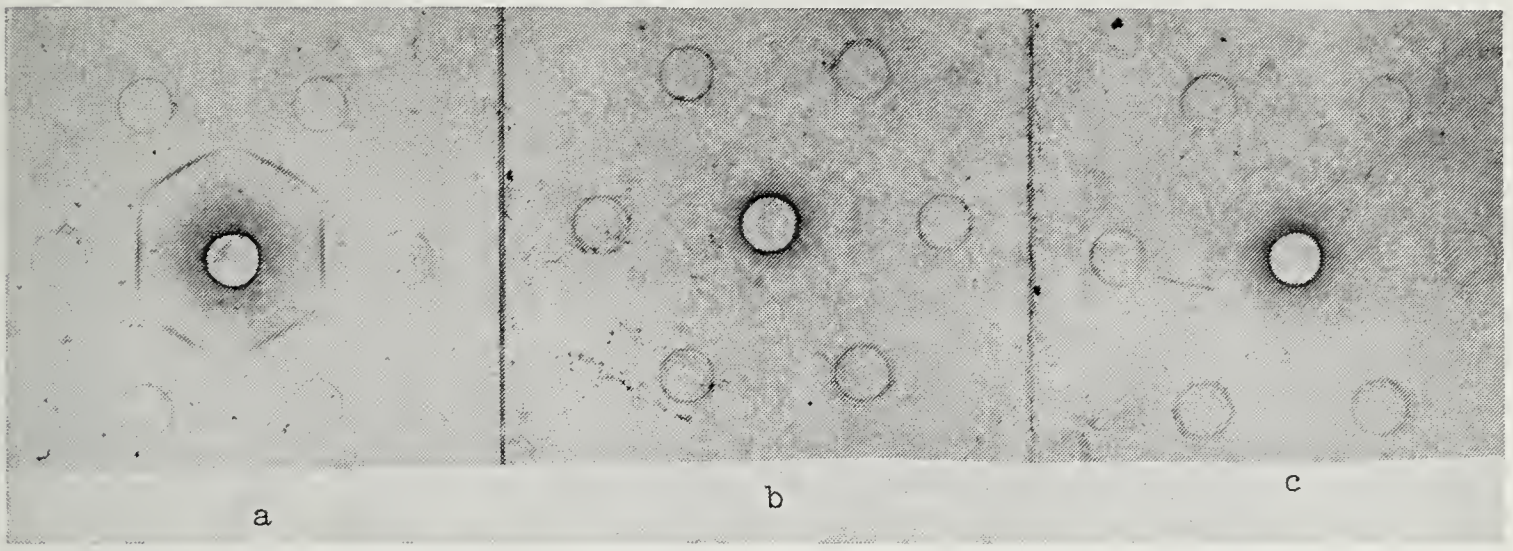

FIGURE 3.—Immunodiffusion Patterns of Fraction LPS-GP-I. Central wells contain antibodies to whole cell (a), fraction SBC (b), and fraction LPS-GP-I (c).

fusion with the single line formed by fraction LPS-AB (Figure 4). Although the immunochemical nature of the second line in fraction LPS-GP-I has not been determined, it has been suggested that it might be formed by the O-specific side chain component of the lipopolysaccharide (Wober and Alaupovic, 1971). These results clearly indicated that fraction LPS-GP-I is immunogenic and shares a common antigenic determinant with fraction LPS-GP-II and the water-soluble LPS. Two important conclusions can be derived from these results. First, fraction LPS-GP-I is not only chemically but also immunochemically similar to the water-soluble lipopolysaccharide (fraction LPS-AB). Secondly, the presence of a common antigenic site in a highly proteinous fraction such as fraction LPS-GP-II may suggest that the protein moiety in the endoxtin molecule is possibly covalently linked. Homma and his coworkers (Homma, *et al.*, 1966) have demonstrated that endotoxins isolated from autolysates of *Pseudomonas aeruginosa* contain antigenic determinants in the polysaccharides as well as in the protein moiety. Similar immunochemical experiments with fraction LPS-GP-II may reveal if the protein moiety in this fraction is immunogenic or not.

Although electrophoretic and immunochemical evidences point to the common nature of the phenol-soluble and water-soluble lipopolysaccharide fractions, further degradative studies will be necessary to explain the distribution of the LPS fractions into two solvent systems. It has been well established (Muller-Seitz, *et al.*, 1968) that the lipid fraction (lipid A) of the lipopolysacchride can be readily dissociated by mild acid hy-

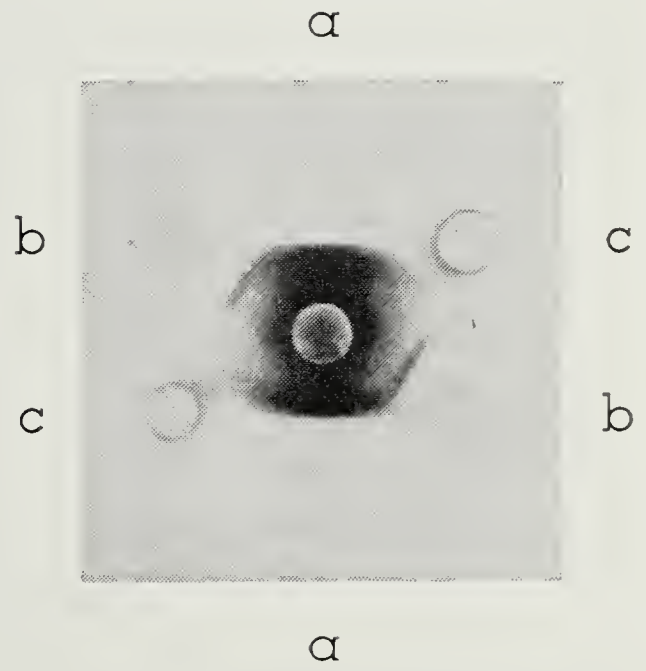

FIGURE 4.—Immunodiffusion Patterns of Cross-Reactions of Fraction LPS-GP-I (a) with Fraction LPS-AB (b), Fraction LPS-WPB (c).

Central well contains antibodies to whole cell of *S. marcescens* Bizio.

drolysis. By the application of centrifugation and followed by gel filtration of the supernate on Sephadex G-50, it is possible to isolate from the hydrolysate separately the lipid A fraction, the polysaccharide O-specific side chain, and the polysaccharide core of the LPS. Chemical and immunochemical characterization of these fractions may provide further information for the understanding of the distribution of LPS between phenol and water solvent systems.

Acknowledgment

The authors wish to express their appreciation for the financial support for this investigation from Research Corporation, Chicago, Illinois, and Illinois State University Research Committee.

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Manuscript received December 9, 1971.

DISTRIBUTION OF HELMINTH PARASITES IN TURTLES NATIVE TO SOUTHERN ILLINOIS

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ABSTRACT.—From October, 1965, through October, 1966, 287 turtles representing six genera and seven species were collected from 31 sites in southern Illinois. These turtles were killed and examined for helminth parasites. Two hundred thirty-two (80.8%) of the turtles were infested with parasites. Twelve genera and 22 species of parasites were identified. The distribution of the various parasites was dependent on food| habits and habitat of host in relation to life cycle of the parasite involved. Eight new host records occurred.

From October, 1965, through October, 1966, 287 turtles representing seven species were collected within a 30 mile radius of Carbondale, Illinois. Species studied in this survey were *Chelydra serpentina, Chrysemys picta, Pseudemys scripta, Sternotherus odoratus, Terrapene carolina, Terrapene ornata,* and *Trionyx spinifer.*

The purposes of this investigation were to determine which helminth parasites were present in the turtles, to determine which species of turtles were parasitized, to determine the degree of infestation, and to determine possible factors affecting the distribution of the helminth parasites in the turtles.

Monogenetic and Digenetic Trematodes, Cestoda, Acanthocephala, Nematoda, and Hirudinea have been reported previously from turtles in North America. However, very little concerning the parasites of turtles found in southern Illinois has been published. Cahn (1937) in his monograph on turtles of the state makes brief mention of finding a few parasites in his survey. The most recent work on turtles of Illinois is summarized in Smith (1961), but little mention is made of parasites. Apparently, few parasites have been reported from turtles in southern Illinois; and no distributional study of the parasites in turtles from this area has been made.

MATERIALS AND METHODS

In this survey, 287 turtles were collected from various sites in southern Illinois and were examined for ecto- and endoparasites. The turtles were collected by hand, by electroshocking, with a hook and line, and with traps.

The turtles were killed and examined for parasites as soon as possible after collection. Any parasites found while examining the external surfaces or internal organs were removed and placed in a 0.75% saline solution.

Trematodes, acanthocephalans, nematodes, and leeches were killed and fixed with either AFA or Gilson's solution. The worms were flattened during fixation by placing them between glass slides ,a slide and a coverslip, or two glass plates. The worms were then transferred to 70% alcohol and stored until staining. Leeches were relaxed before fixation by using a magnesium sulfate solution.

The helminths were stained with borax carmine if the fixative was AFA and with carm alum if the fixative was Gilson's solution. Then, the worms were dehydrated in an alcohol series, cleared in xylene, and mounted in permount.

The turtles were identified with keys and descriptions in Smith (1961). The helminth parasites were identified with keys and descriptions in Acholonu (personal communication), Byrd (1939), Cable and Hopp (1954), Fisher (1960), Mackin

(1936), Pennak (1953), Price (1939), Wharton (1940), Yamaguti (1958, 1961), and Yorke and Maplestone (1926). Verification of the identifications was made by Dr. George Garoian and Miss Lee Morin of the Zoology Department, Southern Illinois University.

Results

Twelve genera and 22 species of helminth parasites were identified. The names of the various parasites and their frequency of occurrence within the various hosts are given in Table I and Table II.

No new species of helminths were found in this survey. However, eight new host records occurred; and these are given in Table III.

Discussion

There is a great deal of variation in the parasitism of the different species of turtles. (Table II). Two species showed no parasites, and one species was 100% infested. The lack

Table I
FREQUENCY OF PARASITES IN SEVEN SPECIES OF TURTLES FROM SOUTHERN ILLINOIS

	Hosts							
	Chelydra serpentina	*Chrysemys picta*	*Pseudemys scripta*	*Sternotherus odoratus*	*Terrapene carolina*	*Terrapene ornata*	*Trionyx spinifer*	TOTALS
Number of hosts examined	17	69	125	10	63	2	1	287
	Number of hosts infested							
Trematoda Monogenea								
Neopolystoma orbiculare		6	11					17
Polystomoides coronatum	1	5	38					44
Digenea:								
Heronimus mollis	5	8	2					15
Spirorchis elegans		2	1					3
Spirorchis magnitestis	2							2
Spirorchis sp.		1	2					3
Telorchis corti	4		2		2			8
Acanthocephala:								
Acanthocephalus sp.	1							1
Neoechinorhynchus chrysemydis		4						4

TABLE I
FREQUENCY OF PARASITES (Continued)

	Hosts							
	Chelydra serpentina	*Chrysemys picta*	*Pseudemys scripta*	*Sternotherus odoratus*	*Terrapene carolina*	*Terrapene ornata*	*Trionyx spinifer*	TOTALS
Neoechinorhynchus emyditoides			26					26
Neoechinorhynchus pseudemydis	1	1	113					115
Neoechinorhynchus sp.			5					5
Nematoda:								
Camallanus trispinosus	4	11	87	1	7			110
Immature nematodes					2			2
Immature oxyuroids	1		2		15			18
Oswaldocruzia leidyi					7			7
Oswaldocruzia pipiens					3			3
Spironoura affine			1		6			7
Spironoura chelydrae	1							1
Spironoura longispicula					1			1
Spironoura sp.	3		1		11			15
Spiroxys contorta	3	10	22		11			36
unidentified nematodes	1		6		3			10
Hirudinea:								
Placobdella parasitica	1	6	6					13
Placobdella rugosa		1	1					2

TABLE II

DEGREE OF HELMINTH INFESTATION OF SEVEN SPECIES OF TURTLES FROM SOUTHERN ILLINOIS

Host species	Number examined	Number parasitized	Percentage parasitism	Number of turtles infested				
				Monogenea	Diginea	Acanthocephala	Nematoda	Hirudinea
Chelydra serpentina	17	14	82.4	1	11	2	9	1
Chrysemys picta	69	42	61.8	12	11	1	20	8
Pseudemys scripta	125	125	100.0	45	7	123	99	12
Sternotherus odoratus	20	2	20.0	0	0	0	2	0
Terrapene carolina	63	48	76.2	0	2	0	48	0
Terrapene ornata	2	0	0.0	0	0	0	0	0
Trionyx spinifer	1	0	0.0	0	0	0	0	0
TOTALS	287	232	80.8	68	31	126	196	21

TABLE III

NEW HOST RECORDS

Parasite	New Host
Acanthocephala:	
Acanthocephalus sp.	*Chelydra serpentina*
Neoechinorhynchus pseudemydis	*Chelydra serpentina*
Nematoda:	
Camallanus trispinosus	*Terrapene carolina*
Oswaldocruzia pipiens	*Terrapene carolina*
Spironoura affine	*Pseudemys scripta*
Spiroxys contota	*Terrapene carolina*
Hirudinea:	
Placobdella parasitica	*Pseudemys scripta*
Placobdella rugosa	*Pseudemys scripta*

of parasites in *T. ornata* and *T. spinifer* was due to the small number of hosts examined, since a variety of parasites has been reported previously from these two species (Barker, 1911; Barker and Parsons, 1914; Harwood, 1932; Hill, 1941; Hughes, Baker, and Dawson, 1941; Hughes, Higginbothan, and Clary, 1942, Morgan, 1945; Odlaug, 1956; Price, 1939; Rausch, 1947; and Stunkard, 1926).

The 100% infestation of *P. scripta* is due to several factors. One of these is diet. They eat molluscs, small fish, insects, crustaceans, and vegetation—all of which are important in the life cycles of various parasitic groups.

Secondly, *P. scripta* is more aquatic in habit (Smith, 1961). There is more opportunity for infestation since the larval stages of most parasitic groups are aquatic or require an aquatic intermediate host (Rankin, 1945).

Table II also shows that the percentage of infectation of two other aquatic turtles — *C. serpentina* and *C. picta* — was relatively high. Again diet and aquatic habit are the major factors for this high percentage.

However, the percentage for *S. odoratus,* also an aquatic turtle, was low — only 20%. The cause of this was not determined. It was probably due to the small number of hosts examined.

Rankin (1945) also noted that a larger variety of parasites occurred in the aquatic habitat than in the terrestrial habitat. Thus, an aquatic turtle should be infested with a greater number of species of parasites than a terrestrial turtle. This can be seen by examining Table I. Although there were ten or more species of parasites found in *P. scripta, C. serpentina, C. picta,* and *T. carolina;* in *T. carolina,* a terrestrial animal, all the parasites (with one exception) were nematodes. The parasites of the other turtles included trematodes, nematodes, acanthocephalans, and leeches.

The species of parasites found in aquatic turtles are usually not the same as those found in terrestrial turtles. An examination of the distribution of each parasitic group in Table I will support this statement.

Monogenetic trematodes normally are restricted to aquatic hosts. This is due to a direct life cycle with an aquatic larval stage. Tables I and II show that monogenetic trematodes were restricted to aquatic hosts in this survey.

Three genera of digenetic trematodes were found in this survey. *Spirorchis* and *Heronimus* were genera that were limited to aquatic hosts (Table I). These genera are limited to aquatic hosts because *Spirorchis* cercaria must directly penetrate the final host (Olson, 1967), and *Heronimus* cercaria require a molluscan intermediate host (Crandall, 1960). *Telorchis,* the third genus, was found in both aquatic and terrestrial turtles. (Table I). Apparently, *Telorchis* has little host specificity as it has been found in many aquatic and terrestrial turtles (Hughes, et. al., 1942; Warton, 1940).

Acanthocephala normally are not restricted to aquatic hosts, as they were in this survey. Since the life cycles of *Neoechinorhynchus chrysemydis, N. emyditoides,* and *N. pseudemydis* have not been completed, it is not yet known if these species can utilize a terrestrial host or if they are limited to aquatic hosts.

Nematodes were the parasites most frequently found in this survey. They infested 62% of the turtles examined. Four genera were identified: *Camallanus, Spironoura, Spiroxys,* and *Oswaldocruzia.*

Oswaldocruzia was limited to *T. carolina,* a terrestrial turtle (Table I). The eggs of this parasite are deposited on the ground and swallowed

accidently while the host is feeding (Baer, 1951). Aquatic turtles normally would not be infested. *Spiroxys* was found only in aquatic turtles (Table I). Copepods serve as intermediate hosts (Baer, 1951), so terrestrial turtles should not be infested. *Camallanus* and *Spironoura* were found in both aquatic and terrestrial turtles (Table I). Fish or copepods serve as intermediate hosts for *Camallanus* (Baer, 1951; Cheng, 1964). This life cycle infers aquatic final hosts. The infestation of *T. carolina* suggests either additional intermediate hosts or an aquatic diet for *T. carolina*. *Spironoura* has a direct life cycle in which eggs can be deposited on land or in water (Cheng, 1964). Both aquatic and terrestrial hosts are possible.

In all previous reports of leeches on turtles, (Cahn, 1937; Mathers, 1948; and Pennak, 1953) only aquatic hosts were involved. The same results were found in this survey.

No tapeworms were found in this survey, although they were reported previously from the same hosts by Cahn, 1937; Hughes, Baker, and Dawson, 1941; and Odlaug, 1956. Their absence in this survey was not determined.

No larval stages of helminth parasites were found. Their absence indicates the turtles are not suitable as intermediate hosts for these parasites.

Summary

As a group, turtles in southern Illinois are parasitized rather heavily. Two hundred thirty-two (80.8%) turtles had helminth infestations. Approximately 20.6% were parasitized by Monogenetic trematodes, 10.8% by Digenetic trematodes, 43.5% by Acanthocephala, 62% by Nematoda, and 7.3% by Hirudinea. The helminth fauna of aquatic turtles was havier and more varied than the helminth fauna of terrestrial turtles from the same area. The distribution of the parasites in the turtles examined infers a relationship between food habits and habitat of host and the life cycle of the parasite involved.

Acknowledgments

The author wishes to acknowledge and to thank Dr. George Garoian for his encouragement, suggestions, and criticisms; Dr. Lee Morin for technical assistance; and all the people who helped in collecting turtles.

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Manuscript received February 21, 1972.

IMMUNOCHEMICAL AND ELECTROPHORETIC CHARACTERIZATION OF PHENOL-SOLUBLE COMPONENTS FROM THE CELL WALL OF *SERRATIA MARCESCENS* BIZIONO

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ABSTRACT.—Phenol-extraction method of Westphal was applied to the whole cells of *Serratia marcescens,* Bizio. An ethanol-precipitable material (fraction WP) was isolated from the phenolic phase. The lipid-free fraction (fraction LFWP) was found to consist mainly of protein (80.8%) and carbohydrate (12%). The heterogeneity of the proteinous material was shown by polyacrylamide gel electrophoresis, and the presence of a glycoprotein was manifested by the periodic acid-Schiff staining. Attempts to isolate the glycoprotein in pure form were unsuccessful. Immunochemically, fraction LFWP possessed a common antigenic determinant with the sodium dodecyl sulfate extracted cell wall glycoprotein and the phenol-soluble components from the endotoxin of the same bacterial strain.

Aqueous phenol extraction method (Westphal *et al.,* 1952) has long been shown to be of high efficiency in fractionation of bacterial cell wall materials. The treatment yields phenol and aqueous soluble fractions. Lipopolysaccharides (LPS) isolated from the aqueous phase have been studied in detail and shown to constitute mainly the cell wall endotoxin complex (LPS-protein complex). However, the components in the phenolic phase have not yet been studied extensively.

When the cell walls of *pseudomonas aeruginosa* were treated with aqueous phenol (Clarke *et al.,* 1967) a methanol-precipitable material was isolated from the phenol layer whose composition was described as almost entirely protein. When this technique was applied to the endotoxin from *Serratia marcescens* 08 and *Escherichia coli* 0 141:K 85 (B) (Wober and Alaupovic, 1971), the ethanolprecipitable material was found to consist of the protein moiety of the LPS-protein complex and a small segment of lipid A. Similar extraction with phenol of *Escherichia coli* B led to the isolation of a material from the phenol-soluble fraction, which was shown to be glycoprotein in nature (Okuda and Weinbaum, 1968).

This report represents the study of the immunochemical and electrophoretic behaviors of the materials isolated from the phenol layer after the whole cell or isolated cell wall of *Serratia marcescens,* Bizio was extracted with aqueous phenol.

MATERIALS AND METHODS

Serratia marcescens, Bizio grown on an inorganic medium was supplied by General Biochemicals, Chagrin Falls, Ohio. The cells were harvested at the late log phase. Wet cells were treated with 45% aqueous phenol for 30 minutes at 65-70 C (Westphal *et al.,* 1952). To the water-washed (three times) phenolic phase 95% ethanol was added in a ratio of 9.5:1 (ethanol:phenol) (Wober and Alaupovic, 1971; Tsang, 1971). The mixture was allowed to stand overnight. The precipitate (fraction WP) was isolated by centrifugation at 10,000 rpm for 15 minutes. After washing with ethanol and acetone fraction WP was submitted to chloroform-methanol 2:1, v/v) extraction in a Soxhlet apparatus. The lipid-free residue was dried and designated fraction LFWP. Carbohydrate content of

the fraction LFWP was estimated by use of the anthrone method (Koehler, 1952) with beta-D-glucose as standard. Protein was measured with bovine serum albumin as standard (Lowry *et al.*, 1951). Hexosamine estimation was performed using beta-D-glucosamine as standard (Rondle and Morgan, 1955). Thin-layer chromatographic analysis (TLC) for neutral sugars was performed on Cellulose plates (Raadsveld and Klomp, 1971) with the following solvent system: freshly distilled ethyl acetate-pyridine-distilled water (100: 35:25, v/v/). The spots were detected with anilinium phosphate in 70% ethanol. Amino acid components were separated on Silicia gel H plates using the following solvent systems: chloroform-methanol-17% ammonium hydroxide (2:2:1, v/v/v) for first dimension and phenol-distilled water (75:25, w/w) for second dimension (Kellerman *et al.*, 1970). Spots were made visible by ninhydrin (0.2%) in acetone spray. TLC for extracted free lipids was also run on Silica gel H plates. The following solvent system was employed: chloroform-methanol-concentrated ammonium hydroxide (75:25: 4, v/v/v). Spray used was molybdenum blue stain. Paper chromatographic analysis was carried out on Whatman No. 1 paper by the descending method. The developing solvent system used was ethyl acetate-pyridine-distilled water (3:6:1.0:1.5, v/v/v). Neutral sugars were detected with ammoniacal silver nitrate spray and amino sugars with ninhydrin spray.

Polyacrylamide gel electrophoresis was performed on 7.5% acrylamide in 8M urea, pH 9.5 at 5 mA per tube for 1 hour according to the modified procedure of Davis (Davis, 1964). The gel columns were stained separately with 0.25% Coomassie blue for protein and with periodic acid-Schiff carbohydrate stain (Zacharius *et al.*, 1969).

Double diffusion immunological studies were performed on microslides (Ouchterlony, 1962), and a solution of 1% agar in 0.05M Veronal buffer, pH 8.6, was used as the supporting medium. For immunoelectrophoresis the same supporting medium was used. Electrophoresis was run for 45 minutes at 10 mA per agar-covered rack in a Gelman Deluxe electrophoresis chamber and gel diffusion precipitation was performed (Ouchterlony, 1962). The slides were stained with amido black for better visualization.

Fraction LFWP was fractionated by column chromotography on Sephadex G-200 gel and Sephorose 6B. Sample was solubilized by 0.05M tris-HCI buffer, pH 7.6, containing 0.5% sodium dodecyl sulfate (SDS). Columns were eluted by the same buffer and fractions of 3 ml were collected Aliquots of 0.2 ml were taken for protein analysis (Lowry *et al.*, 1951).

Results and Discussion

When the cell walls of *Pseudomonas aeruginosa* (Clarke *et al.*, 1967) were extracted with aqueous phenol, a menthanol-precipitable protein was obtained from the phenol layer with a yield of 24.5% of the cell walls. However, when the intact cells or isolated cell wall of *Serratia marcescens*, Bizio were submitted to the same treatment, the yields of the ethanol-precipitable material (fraction WP) from the phenolic phase were 7.3% (based on the dry whole cells) and 15% (based on cell wall), respectively. The amount of extractable lipids was 3.2% of fraction WP, in which alpha-lecithin and phosphotidyl ethanolamine were detected by TLC. The lipid-free fraction (fraction LFWP) was found to consist mainly of protein (80.8%) and carbohydrate

(12%). Tsang and co-workers (Tsang *et al.*, 1971) reported a sodium dodecyl sulfate (SDS)-extracted glycoprotein from the same bacteria, which contained less protein (58%) and higher lipid (33%). This may indicate that SDS is milder, less effective, and also less selective in protein extraction from the outer layer than phenol (Pusztai, 1966.

A total of 13 common amino acids and 3 unidentified spots were detected by TLC in fraction LFWP. They were similar to those obtained from the cell wall protein of *Brucella abortus* (Kellerman et al., 1970). The carbohydrate components of the extract (fraction LFWP) were identified as mainly glucose and glucosamine. Surprisingly, trace amount of mannose, galactose, and even rhamnose were also observed. The existence of these sugars may suggest the possible contamination by any phenol-soluble lipopolysaccharide (Raff *et al.*, 1968; Tsang, 1971).

Fraction LFWP was purified by gel filtration on a Sephadex G-200 column. Although three peaks were obtained, the resolution was poor. When Sephrose 6B was used, the separation was improved but far from satisfactory (Figure 1). The three peaks were designated 6B-G-I, 6B-G-II, and 6B-G-III, respectively. It is hoped that ion-exchange chromatographic technique will give better separation.

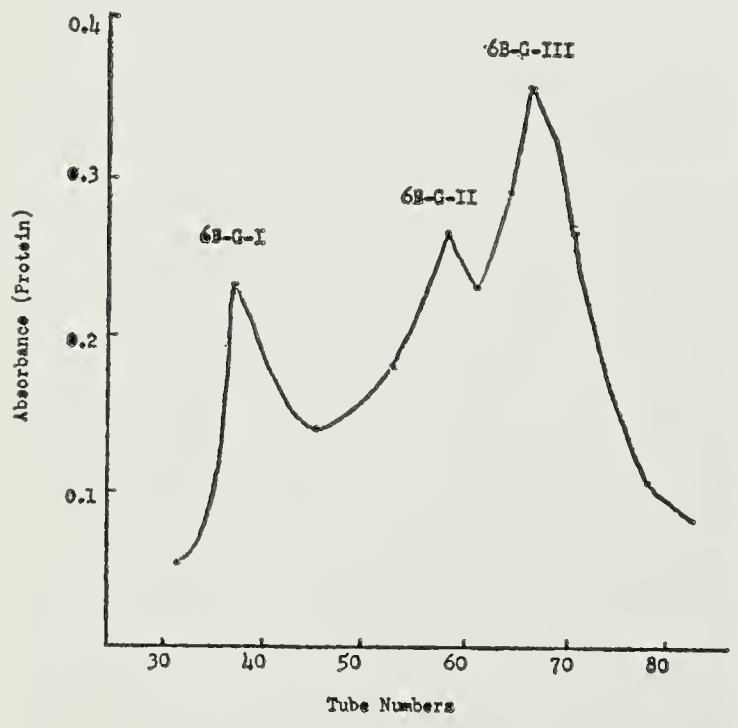

FIGURE. 1—Fractionation of Phenol- soluble Proteins from Cell Walls of *Serratia marcescens*, Bizio on Sepharose 6B.

On polyacrylamide gel electrophoresis fraction LFWP gave three discrete protein bands with Coomassie blue, which were also positive with the periodic acid-Schiff staining procedure (Zacharius *et al.*, 1969). However, after fraction LFWP was washed exhaustively with water, only one carbohydrate band was observable while all three protein bands were retained. It is tempted to suggest that the ethanol-precipitable material may contain at least one component which is glyco-protein in nature. Upon gel electrophoresis fraction 6B-G-I migrated as a single diffused protein band, while both of fractions 6B-G-II and 6B-G-III gave four bands. All these three subfractions showed positive reaction with periodic acid-Schiff stain and gave the same carbohydrate band as observed in the washed fraction of LFWP. This electrophoretic study confirms the presence of at least one glycoprotein in the cell wall which had been suggested previously (Tsang *et al.*, 1971).

The antigenicity of the phenol extracted proteineous material (fraction LFWP) and its subfractions from Sepharose 6B (fractions 6B-G-I, 6B-G-II, and 6B-G-III) were characterized by means of immunodiffusion and immunoelectrophoresis. Fraction LFWP formed three precipitin lines with anti-whole cell serum in both types of analysis (Figure 2, pattern 1 and Figure 3, pattern 1). The same fraction showed one line in cross-reaction with the SDS-soluble glycoprotein (fraction S-SB) using the antiserum (anti-S-SBC) to the purified

fraction S-SBC (Tsang *et al.*, 1971) (Figure 2, pattern 2). On agar immunoelectrophoresis, fraction LFWP and fraction S-SB demonstrated similar mobility (Figure 3, pattern 2). It is possible that these two different preparations from the same bacteria may share a common antigenic component. From a previous study (Tsang, 1971), the phenol-soluble components from endotoxin of *Serratia marcescens*, Bizio were shown to be a mixture of phenol-soluble lipopolusaccharide (fraction LPS-GP-I) and lipoglycoprotein (fraction LPS-GP-II). When fraction LFWP was allowed to react with antibodies prepared against fraction LPS-GP-I (anti-GP) two lines were detected (Figure 2, pattern 3). A similar result was obtained on immunoelectrophoresis (Figure 2, pattern 3). The antigenic specificity of fraction LFWP and its cross-reactivity with both fractions

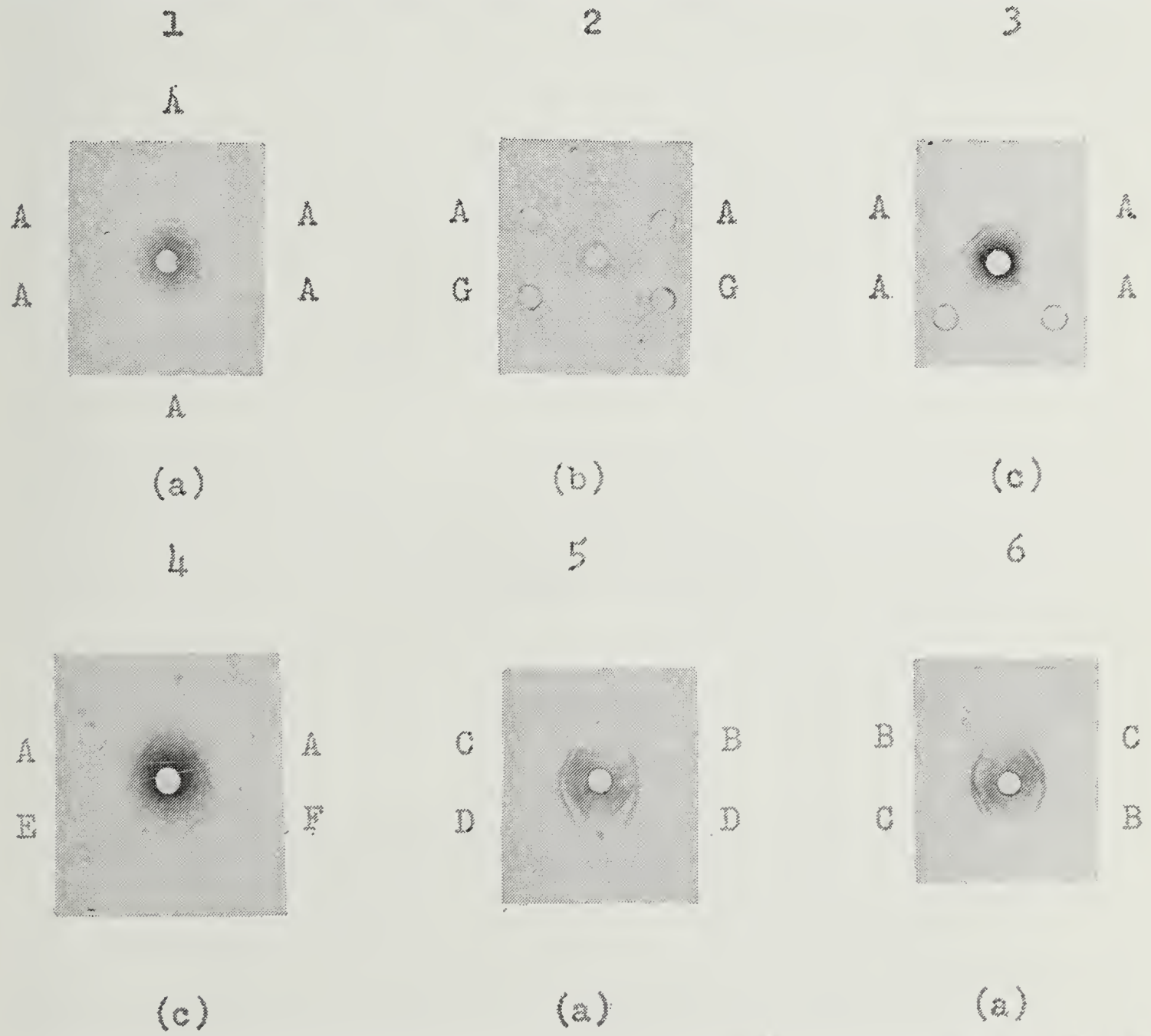

FIGURE 2.—Immunodiffusion Patterns of Fraction LFWP and Its Subfractions from Sepharose 6B.

Antibodies contained in the central wells:
(a) against whole cells, Bizio
(b) against fraction S-SBC
(c) against fraction LPS-GP-I

Antigens applied:
A: fraction LFWP
B: fraction 6B-G-I
C: fraction 6B-G-II
D: fraction 6B-G-III
E: fraction LPS-GP-I
F: fraction LPS-GP-II
G: fraction S-SB

LPS-GP-I and LPS-GP-II are shown in Figure 2, pattern 4. It is interesting to note that the antigenic determinant common to the two phenol-soluble fractions from the endotoxin may also be shared by the phenol-soluble protein from the cell walls. Indeed, a similar cell wall protein from *Pseudomonas aeruginosa* was also shown to cross-react with the protein moiety of endotoxin (Homma and Suzuki, 1964).

On immunodiffusion, subfractions 6B-G-II and 6B-G-III gave three precipitin lines against anti-whole cell serum while subfraction 6B-G-I gave only two. However, a common antigenic component is present in all

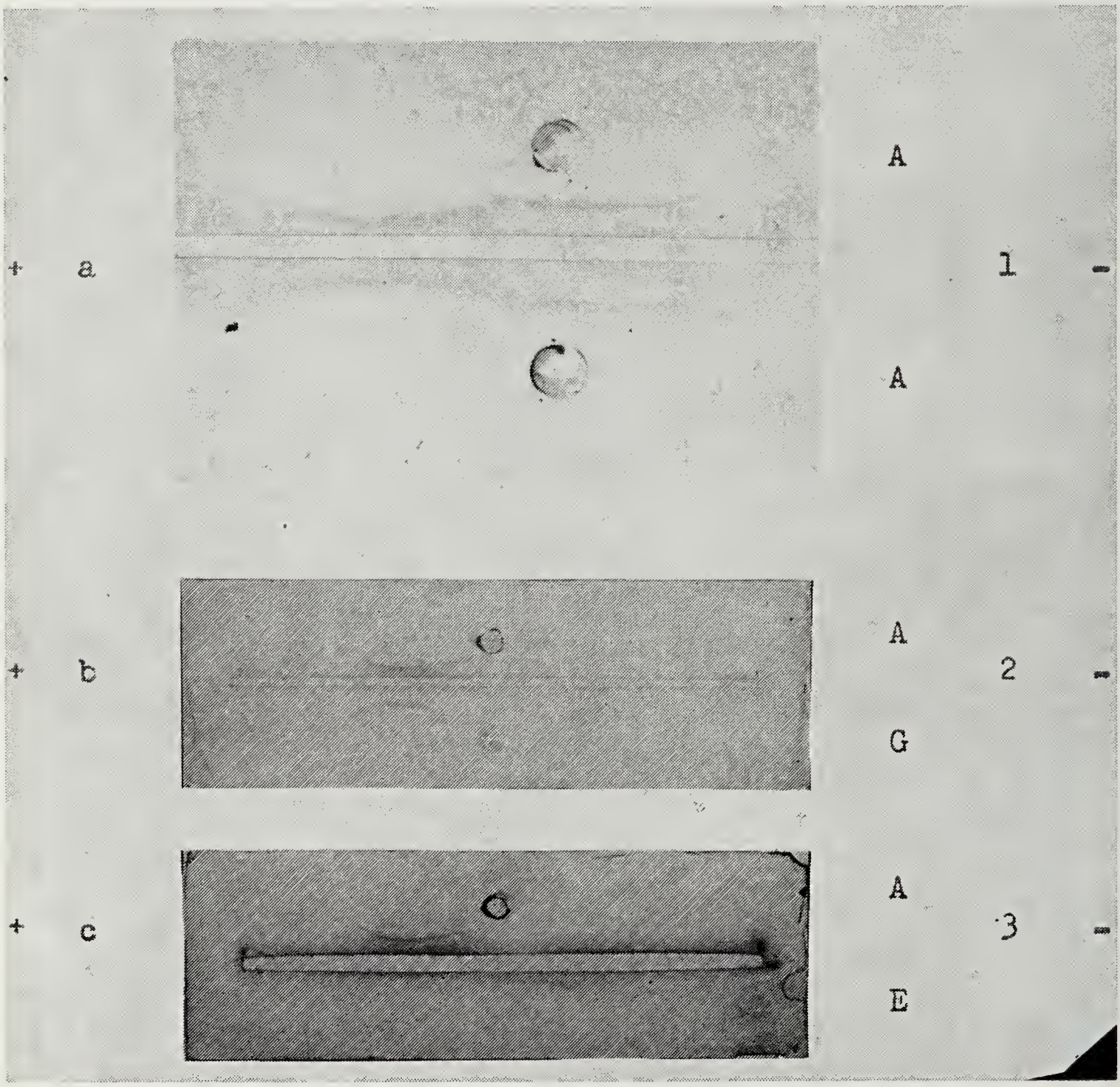

FIGURE 3.—Immunoelectrophoresis Patterns of Fraction LFWP.

Antibodies contained in the three central troughs:

a: against whole cells, Bizio
b: against fraction S-SBC
c: against fraction LPS-GP-I

Antigens applied:

A: fraction LFWP
G: fraction S-SB
E: fraction LPS-GP-I

three fractions (Figure 2, patterns 5 and 6). On immunoelectrophoresis against anti-GP, two identical components with varied intensities were present in all three fractions (Figure 4, patterns 1, 2, and 3). These results demonstrate the common antigenic property and the immunochemical heterogeneity of the subfractions.

Hot aqueous phenol extraction has revealed valuable information about the outer surface structure of Gram-negative bacteria; but when the intact cells were used, the source of fractions cannot be certain. Therefore, in separate experiments, isolated cell walls instead of the whole cells were used for extraction. The ethanol-precipitable material was shown to be similar to fraction LFWP chemically (79% protein, 10.3% carbohydrate), electrophoretically (3 components), and immunochemically (3 components against anti-whole serum, 2 components against anti-phenol-soluble LPS, and 1 component against anti-SDS extractable glycoprotein).

From the present electrophoretic

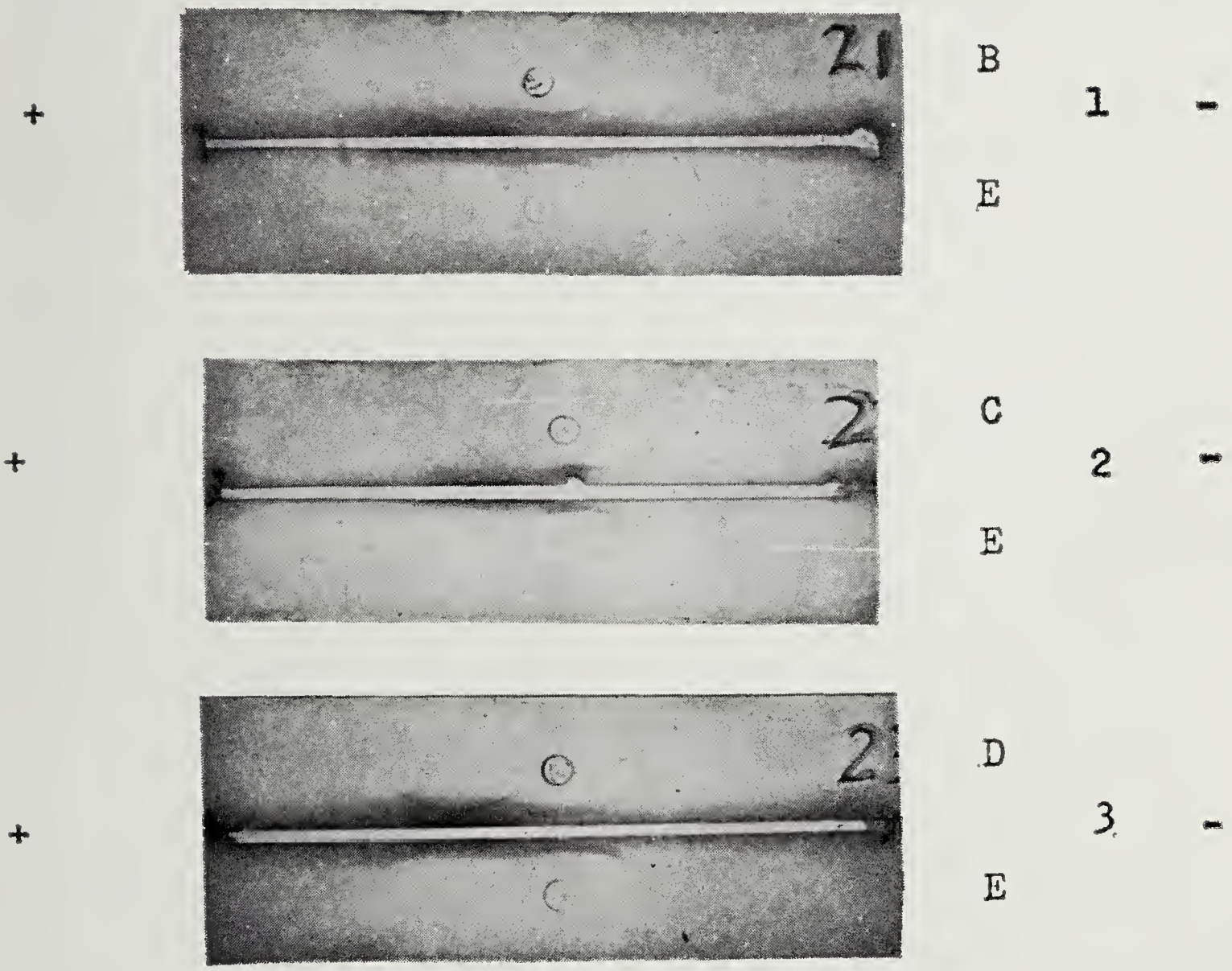

FIGURE 4.—Immunoelectrophoresis Patterns of Subfractions from Sepharose 6B Column Chromatography.

All troughs contain antiserum against fraction LPS-GP-I
Antigens in the wells:

B: fraction 6B-G-I
C: fraction 6B-G-II
D: fraction 6B-G-III
E: fraction LPS-GP-I

and immunochemical studies two conclusions may be drawn. First, the presence of an antigenic glycoprotein in the phenol-soluble components from the cell wall was confirmed. Secondly, the possible contamination by phenol-soluble lipopolysaccharide in the phenol-phase may present a purification problem for the study of phenol-soluble cell wall glycoprotein.

Acknowledgments

The authors wish to express their appreciation for the financial support for this investigation from Research Corporation, Chicago, Illinois and Illinois State University Research Committee.

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Manuscript received November 1, 1971.

REACTION PRODUCTS OF PENTACHLOROBENZENE WITH POTASSIUM FLUORIDE IN DIMETHYL SULFOXIDE

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ABSTRACT.—Pentachlorobenzene was allowed to react with potassium fluoride in dimethyl sulfoxide at 180° C for 24 hours in a glass vessel. Seven compounds were identified by NMR-^{19}F and infrared spectra: 2,4-dichloro-1,3,5-trifluorobenzene (7.3%), three difluorotrichlorobenzenes (30.3%), and three monofluorotetrachlorobenzenes (17.1%). No hydrogenated or rearranged products were detected in the reaction mixtures. Required reference compounds were synthesized by classical methods. Two new compounds, 1-3-difluoro-2,4,6-trichlorobenzene and 1,3-difluoro-4,5,6-trichlorobenzene, were synthesized.

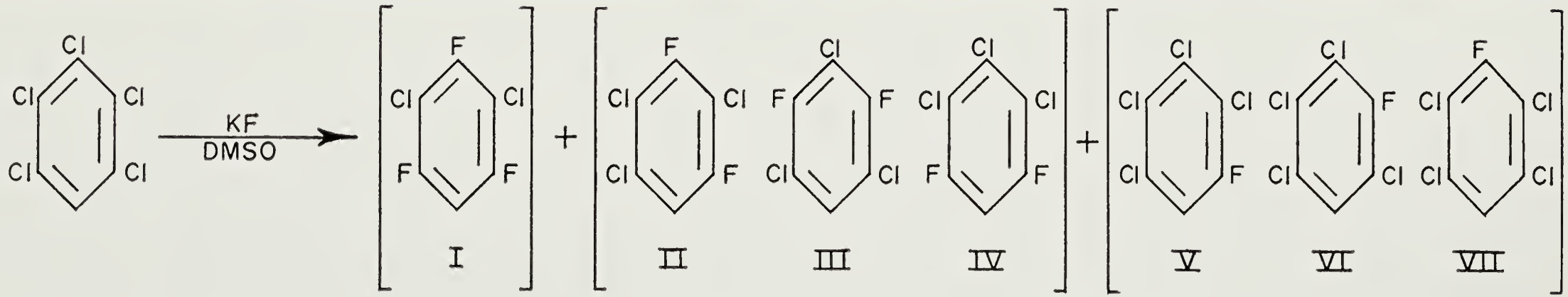
Soon after studies on the replacement of chlorine by fluorine in chlorobenzenes by reaction with potassium fluoride in aprotic solvents were started in our laboratories, we recognized that certain chlorine atoms in 1,2,3,5-tetrachloro-, pentachloro-, and hexachlorobenzenes exhibited sufficient laability to undergo the chlorine-fluorine exchange reaction. Hexachlorobenzene was reported to give 1,3,5-trichlorotrifluorobenzene (Finger et al., 1957). Since then investigators have extended the exchange reaction to many other polychlorinated aromatic compounds.

Yakobson et al. (1966) treated pentachlorobenzene with potassium fluoride without a solvent at 260-390° C for 30 hours in a stainless steel autoclave and obtained a complex reaction mixture. Low yields of six polycholorofluorobenzenes and 3% of pentafluorobenzene were identified in the mixture. Several reductive dehalogenation and rearranged products were also observed. No literature data are available on the effects of less drastic conditions.

In our work, pentachlorobenzene was treated with potassium fluoride in dimethyl sulfoxide at 180° C for 24 hours. Distillation of the resulting mixture gave three fractions indicating different degrees of halogen replacement (See Table I). In general, nuclear magnetic resonance (NMR-^{19}F) spectra were used to identify the principal components of the fractions. Fraction **A** was identified as 1,3-dichloro-2,4,6-trifluorobenzene (**I**). Fraction **B** was a mixture (approximate ratio 2:1:1) of 1,3-difluoro-2,4,5-trichlorobenzene (**II**), 1,3-difluoro-2,4,6-trichlorobenzene (**III**), and 1,3-difluoro-4,5,6-trichlorobenzene (**IV**). Fraction C was a mixture of approximately equal parts of 2,3,4,5-tetrachlorofluorobenzene (**V**), 2,3,4,6-tetrachlorofluorobenzene (**VI**), and 2,3,5,6-tetrachlorofluorobenzene (**VII**). No products resulting from reductive dehalogenation or rearrangement were isolated. In summary, the mild reaction conditions resulted in a 55% molar yield of a mixture consisting of three monofluoro isomers (C_6HCl_4F), three difluoro isomers ($C_6HCl_3F_2$), and one trifluorobenzene ($C_6HCl_2F_3$).

Studies of the reaction of potassium fluoride with hexachlorobenzene by Holbrook et al. (1966) indicated that the directive effect of fluorine is primarily meta, which we also observed in this study on pentachlorobenzene. In each di- and trifluorinated product (**I-IV**) the fluorine atoms are meta with respect to each other.

TABLE 1.—Fluorination of Pentachlorobenzene



DISTILLATION FRACTION	A	B			C		
FRACTION b.p. (°C/mm)	79-82/42		89/15		114/8.5		
COMPONENTS b.p. (°C/mm)	160/atm	91/45	104/18	200/atm	75/0.7	---	---
FRACTION n_D^{20}	1.4870		1.5298			1.5738	
COMPONENTS n_D^{20}	1.4865	1.5310	1.5290	---	---	---	---
COMPONENTS m.p. (°C)	---	---	---	50	65-66 a/	17 b/	71.5-72.5 c/
YIELD OF FRACTION (%)	7.3		30.3			17.1	
DISTRIBUTION (%)	100	50.0	25.0	25.0	33.3	33.3	33.3

a/ Lit. m.p. 63-64°C (Vorozhtsov et al., 1961a).
b/ Germano and Sechaud, 1954.
c/ Finger and Kruse, 1956.

Experimental

The potassium fluoride used in this experiment was dried at 120° C for 24 hours and quickly powdered in a warm mortar.

Melting and boiling points are uncorrected.

The NMR-^{19}F spectra were run using a Varian DP-60 instrument. Interpretations of the spectra were made according to Gutowsky et al. (1952) using fluorobenzene as an external standard.

Fluorination of Pentachlorobenzene. — A mixture of 33.5 g (0.137 mole) of pentachlorobenzene, 58.0 g (1 mole) of finely ground potassium fluoride, and 100 ml of dimethyl sulfoxide (DMSO) was dried by azeotropic distillation with benzene, and then stirred and heated at 180° C for 24 hours. The crude product was diluted with water and steam distilled. Vacuum distillation gave three fractions: **A**, 2.0 g (7.3%), b.p. 61-63° C (23 mm), n_d^{20} 1.4897; **B**, 9.0 g (30.3%), b.p. 61-83° C (17 mm), n_d^{20} 1.5300; and **C**, 5.5 g (17.1%), b.p. 81-199° C (17 mm), n_d^{20} 1.5661. Percentages were calculated on the basis of molar yields of products identified below. *Fraction A*: Redistillation gave pure **I**, b.p. 79-82° C (42 mm), n_d^{20} 1.4870.

Anal. Calcd. for $C_6HCl_2F_3$: C, 35.85; H, 0.50; Cl, 35.28; F, 28.36. Found: C, 35.67; H, 0.71; Cl, 35.53; F, 28.50.

2,4 - Dichloro - 3,5 - difluoronitrobenzene (**VIII**). — A Sandmeyer reaction with cuprous chloride on 3-chloro - 2,4 - difluoro - 6 - nitroaniline (Finger et al., 1956), utilizing nitrosyl sulfuric acid and 85% phosphoric acid in the diazotization, and a subsequent steam distillation gave a 66% yield of a yellow oil. Vacuum distillation gave pure **VIII**, b.p. 113° C ((12.5 mm).

Anal. Calcd. for $C_6HCl_2F_2NO_2$: C, 31.61; H, 0.44; Cl, 31.10; N, 6.14. Found: C, 31.80; H, 0.50; Cl, 30.85; N, 6.14.

1,3 - Dichloro - 2,4,6 - trifluorobenzene (**I**). — A mixture of 20.0 g (0.09 mole) of VIII, 11.6 g (0.2 mole) of KF, and 130 ml of DMSO was dried by azeotropic distillation with benzene and then heated at 135° C for 45 minutes until the evolution of the oxides of nitrogen had ceased. The temperature of the mixture was gradually increased to 180° C and then cooled. Steam distillation gave 6.0 g (33%) of a crude yellow oil. Distillation gave pure **I**, b.p. 160° C, n_d^{20} 1.4865. This product was found to be identical with that produced in the "halogen-exchange" reaction when their infrared spectra were compared. Literature data: b.p. 161.5-162° C, n_d^{20} 1.4863 (Finger et al., 1951). *Fraction B:* Purification by vacuum distillation gave a colorless oil, b.p. 89° C (15mm), n_d^{20} 1.5298.

Anal. Calcd. for $C_6HCl_3F_2$: C, 33.14; H, 0.46; Cl, 48.92; F, 17.48. Found: C, 33.06; H, 0.52; Cl, 48.68; F, 17.66.

The NMR-^{19}F spectrum indicated that the sample was a mixture of the three difluoro isomers **II, III,** and **IV** (approximate ratio 2:1:1, respectively).

2,4 - Dichloro - 3,5 - difluoroaniline (**IX**). — A stannous chloride — hydrochloric acid reduction of 32.0 g (0.14 mole) of **VIII** gave a 25.0 g (90%) crude yield of **IX**. Recrystallization from a mixture of benzene and low-boiling petroleum ether, followed by vacuum sublimation, gave pure *IX*, m.p. 65.6-66.5° C.

Anal. Calcd. for $C_6H_3Cl_2F_2N$: C, 36.39; H, 1.53; Cl, 35.81; N, 7.07. Found: C, 36.21; H, 1.63; Cl, 36.09; N, 6.90.

The acetyl derivative was recrystallized from benzene, m.p. 118.5-119° C.

Anal. Calcd. for $C_8H_5Cl_2F_2NO$: C,

40.00; H, 2.08. Found: C, 39.97; H, 2.24.

1,3 - Difluoro - 2,4,5 - trichlorobenzene (**II**). — A Sandmeyer reaction with cuprous chloride on 31.0 g (0.156 mole) of **IX** using nitrosyl sulfuric acid and 85% phosphoric acid in the diazotization gave, after vacuum distillation, 11.0 g (32.5%) of pure **II**, b.p. 91° C (45 mm), n_d^{20} 1.5310. Literature datum: n_d^{21} 1.5308 (Yakobson et al., 1966).

Anal. Calcd. for $C_6HCl_3F_2$: C, 33.14; H, 0.46; Cl, 48.92; F, 17.48. Found: C, 32.89; H, 0.63; Cl, 48.65; F, 17.24.

3 - Fluoro - 2,4,6 - trichloronitrobenzene (**X**). — A mixed acid nitration of 39.9 g (0.2 mole) of 2,4,6-trichlorofluorobenzene (Booth et al., 1935) at 50° C gave a 44.5 g (91.5%) yield of crude steam-distilled **X**. Vacuum distillation gave pure **X**, b.p. 91° C (2 mm), n_{γ}^{25} 1.5585. Literature data: b.p. 216-220° C (atm), n_{γ}^{20} 1.5680 (Kobrina and Yakobson, 1965).

3 - Fluoro - 2,4,6 - trichloroaniline. — A stannous chloride — hydrochloric acid reduction of 10.0 g (0.04 mole) of **X** followed by recrystallization from a mixture of benzene and low-boiling petroleum ether gave 4.3 g (71.5%) of crude product. Vacuum sublimation gave pure 3-fluoro-2,4,6-trichloroaniline, m.p. 69-69.5° C.

Anal. Calcd. for$C_6H_3Cl_3FN$: C, 33.60; H, 1.41; Cl, 49.60; N, 6.53. Found: C, 33.86; H, 1.54; Cl, 49.56; N, 6.67.

The acetyl derivative was recrystallized from benzene and vacuum sublimed, m.p. 193-194° C (corr).

Anal. Calcd. for $C_8H_5Cl_3FNO$: C, 37.46; H, 1.96. Found: C, 37.53; H, 2.11.

1,3 - Difluoro - 2,4,6 - trichlorobenzene (**III**). — A mixture of 15.0 g (0.062 mole) of **X**, 17.8 g (0.31 mole) of KF, and 30 ml of DMSO was heated to 170° C for a period of 2 hours. Evolution of nitrogen oxide fumes was first noted when the temperature reached 150° C. After it cooled, the mixture was poured into water. Steam distillation, followed by vacuum distillation, gave a 2.0 g yield (15%) of pure **III**, b.p. 104° C (18 mm), n_d^{20} 1.5290.

Anal. Calcd. for $C_6HCl_3F_2$: C, 33.14; H, 0.46; Cl, 48.92; F, 17.48. Found: C, 33.36; H, 0.72; Cl, 48.70; F, 17.39.

3 - Chloro - 4,6 - difluoro - 2 - nitroaniline (**XI**). — To a well stirred slurry of 36 ml of glacial acetic acid and 36.5 g (0.18 mole) of 3-chloro-4,6-difluoracetanilide (Finger and Oesterling, 1956) was added 52 ml of concentrated sulfuric acid at 30-40° C. A mixture containing 21 ml (0.33 mole) of 70% nitric acid and an equal volume of concentrated sulfuric acid was added in 15 minutes. The temperature was allowed to rise naturally to 60° C and stirring was continued for 2 hours. The mixture was poured into ice and filtered. The crude nitroacetanilide was hydrolyzed to the nitroamine by heating the mixture with 60 ml of concentrated sulfuric acid at steam bath temperature for 2 hours. The mixture was poured into ice. Recrystallization from 3-fluorobenzotrifluoride and from ethanol followed by a vacuum sublimation gave pure **XI** as orange-yellow needles, m.p. 82-82.5° C.

Anal. Calcd. for $C_{20}H_{11}ClF_2N_2$: C, C, 34.55; H, 1.45; N, 13.43. Found: C, 34.76; H, 1.39; N, 13.27.

The acetyl derivative was recrystallized from ethanol to give white needles, m.p. 184° C.

Anal. Calcd. for $C_8H_5ClF_2NO_3$: N, 11.17. Found: N, 11.15.

To prove the adjacency of the nitro and amino groups, the nitroamine was converted to the diamine and a subsequent reaction with benzil gave

a quinoxaline derivative. Recrystallization from ethanol gave tan needles. Vacuum sublimation gave pure 2,3 - diphenyl - 5 - chloro - 6,8 - difluoroquinoxaline as a white solid, m.p. 179-180° C.

Anal. Calcd. for $C_{20}H_{11}ClF_2N_2$: C, 68.09; H, 3.14; N, 7.94. Found: C, 68.08; H, 3.25; N, 8.09.

2,6 - Dichloro - 3,5 - difluoronitrobenzene (**XII**). — A Sandmeyer reaction with cuprous chloride on 43.0 g (0.19 mole) of **XI**, utilizing nitrosyl sulfuric acid and 85% phosphoric acid in the diazotization, followed by steam distillation gave a 39.0 g (83%) yield of a crude yellow oil. Vacuum distillation gave pure **XII**, b.p. 114° C (20 mm), 228-229° C (microcapillary), m.p. 39-40° C. Literature datum: m.p. 42° C (Vorozhtsov et al., 1961a).

A mixed acid nitration of 1,5-dichloro-2,4-difluorobenzene with fuming sulfuric and fuming nitric acids also gave **XII** in a 68% yield, b.p. 116° C (20 mm). The melting point of a mixture of the two products failed to show a depression.

2,6 - Dichloro - 3,5 - difluoroaniline (**XIII**). — An iron reduction (Lukasevich and Voroshilova, 1935) of 33.0 g (0.145 mole) of **XII** gave 28.0 g (96.5%) of crude **XIII**. Recrystallization from ethanol, followed by vacuum sublimation, gave pure **XIII**, m.p. 77-78° C, b.p. 218-219° C (microcapillary). Literature datum: m.p. 73° C (Vorozhtsov et al., 1961b).

The acetyl derivative was prepared with acetyl chloride as acetylation failed to take place in acetic anhydride. Recrystallization from ethanol gave white needles, m.p. 183° C.

Anal. Calcd. for $C_8H_5Cl_2F_2NO$: N, 5.83. Found: N, 5.78.

1,3 - Difluoro - 4,5,6 - trichlorobenzene (**IV**). — A Sandmeyer reaction with cuprous chloride on 5.0 g (0.025 mole) of **XIII** gave **IV** as a low melting solid. Recrystallization from ethanol and methanol, followed by vacuum sublimation, gave pure **IV**, m.p. 50° C, b.p. 200° C.

Anal. Calcd. for $C_6HCl_3F_2$: C, 33.14; H, 0.46. Found: C, 33.15; H, 0.40.

Fraction C: Vacuum distillation gave a colorless oil, b.p. 114° C (8.5 mm), n_d^{20} 1.5738. The analysis indicated a composition of C_6HCl_4F.

Anal. Calcd. for C_6HCl_4F: C, 30.81; H, 0.43; Cl, 60.64; F, 8.12. Found: C, 31.08; H, 0.40; Cl, 60.42; F, 8.13.

The NMR-^{19}F spectrum of the analytical sample showed it to be a mixture of **V** (Vorozhtov et al., 1961a), **VI** (Germano and Sechaud, 1954), and **VII** (Finger and Kruse, 1956) in equal amounts.

Acknowledgments

The authors thank Dr. H. S. Gutowsky, University of Illinois, for the NMR spectra and his help in their interpretation, and Mr. J. Brader, formerly of the same University, for his help in the interpretation of the infrared spectra. The authors also acknowledge the assistance of Dr. J. L. Finnerty, formerly of the Survey staff. The dimethyl sulfoxide (DMSO) was obtained from the Crown Zellerbach Corporation, Camas, Washington.

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Manuscript received May 28, 1971.

EFFECTS OF THE CHLOROGROUPS OF PHENOXY COMPOUNDS ON THE OXIDATIVE PHOSOHORYLATION

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ABSTRACT.—The effects of the sodium salts of phenoxyacetic acid, phenoxypropionic acid, 2-chlorophenoxyacetic acid (2-CPA), 4-chlorophenoxyacetic acid, (4-CPA), 2,4-dichlorophenoxpropionic acid, dichlorprop, 4(2,4-dichloro phenoxy) butyric acid (2,4-DB) on the oxygen and phosphate uptake by the bluegill (*Lepomis macrochirus*) liver mitochondria in the presence of succinate and alphahitreglutarate as substrates were investigated and compared with the effects of 2,4- dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2-(2,4,5-trichlorophenoxy) propionic acid, silvex. The phenoxyacetic and phenoxypropionic acids did not alter the oxygen uptake in the presence of either substrate. The 2-CPA inhibited oxygen uptake more than the 4-CPA derivative, and the dichloro-substituted phenoxyacetic acid, 2,4-D inhibited oxygen uptake to about the same extent as the 4-CPA. The oxygen uptake was more severely inhibited by the trichloro derivative (2,4,5-T) in the presence of both substrates. The dichlorprop inhibited oxygen uptake more than the trichloro derivative, silvex.

In the presence of succinate, the phenoxyacetic and phenoxypropionic acids increased phosphate uptake, but in the presence of alpha-ketoglutarate, the phenoxyacetic acid and phenoxypropionic derivatives decreased phosphate uptake. The substituted chloro groups did not appear to have an appreciable affect on phosphate metabolism.

While investigating the effects of phenoxy herbicides on energy production by bluegill liver mitochondria, we found that various phenoxy derivatives altered oxidative phosphorylation in the presence of either succinate or alpha-hitoglutarate as substrates. Many of these derivatives altered the hydrolysis of ATP. A specific pattern of effects could not be determined, but the data suggested that the chloro-substituted phenoxy compounds inhibited oxygen uptake by bluegill liver mitochondria, but the effects on oxygen uptake were altered by the various derivatives substituted on the acetic acid.

The sodium salts of phenoxyacetic acid and phenoxypropionic acid did not inhibit oxygen uptake by bluegill liver mitochondria, whereas the sodium salts of the chloro-substituted phenoxy compounds altered the uptake of oxygen. This finding suggested that the uptake of oxygen by bluegill liver mitochondria was influenced by the position and number of chloro groups substituted on the phenoxy ring.

METHODS

Native wild bluegills from various bodies of water in central Illinois were held approximately two weeks at 25° C in aerated laboratory aquaria prior to use. Procedures for the preparation of the liver mitcchondria, for estimating the rates of oxygen and phosphate metabol'sm by conventional Warburg and spectrophotometric techniques, respectively, for estimating the rate of release of inorganic phosphate from the ATP, and for estimating the nitrogen content of the mitcchondrial preparations, have been reported (Hiltibran and Johnson 1965). Oxygen data were converted to microliters of 0_2 per hour per milligram of tissue nitrcgen (ul 0_2/hr/mg N), and all phosphate data were converted to micromoles of PO_4 per hour per milligram for tissue nitrogen (mircomoles/ PO_4/hr/mg N). All data have been corrected for endogenous enzyme activity, and the results presented are the average values from three or more experiments. The sodium salts were

prepared from reference samples, 2,4,5-T and silvex or reagent grade phenoxyacetic acid, phenoxypropionic, 2-CPA, and 4-CPA.

Results and Discussion

The effects of phenoxy compounds on the oxygen and phosphate uptake by bluegill liver mitochondria in the presence of succinate as substrate are summarized in Table 1. The sodium salts of the phenoxyacetic or phenoxypropionic acid did not alter oxygen uptake. The phenoxy compound with one chloro group at the 2 position, 2-chlorophenoxyacetic acid (2-CPA) inhibited oxygen uptake approximately 38 percent, and inhibited oxygen uptake more than the phenoxy compound with one chloro group at the 4 position, 4-chlorophenoxyacetic acid (4-CPA). The phenoxy compound with the chloro groups at the 2 and 4 positions 2,4-dichlorophenoxyacetic acid (2,4-D) inhibited oxygen uptake less than the 2-CPA, but more than the 4-CPA. The phenoxy compound with three chloro groups, at the 2, 4 and 5 positions (2,4,5-T) inhibited oxygen uptake more than the other chloro phenoxy compounds used in this investigation.

Table 1.—Effects of chlorophenoxy compounds on succinic oxidase.

		CONTROL		EXPERIMENTAL	
Derivative	g x 10^{-4} per ml Reaction medium	ulO_2/hr/ mg N (Ave.)	umoles PO_4/hr/ mg N (Ave.) (1)	μl O_2/hr/ mg N (Ave.)	μmoles PO_4/hr/ mg N (Ave.) (1)
Phenoxyacetic	1.5	253	24	253	34
# exp.		(4)	(4)	(5)	(5)
Range		(175-328)	(5-44)	(211-310)	(25-47)
2-chloro	3.4	205	41	125	24
# exp.		(4)	(4)	(4)	(4)
Range		(116-397)	(20 - 74)	(73-256)	(7-46)
4-chloro	3.4	205	41	152	26
# exp.		(4)	(4)	(4)	(4)
Range		(116-397)	(20 - 74)	(51-308)	(16-35)
Dichloro	3.7	310	37	224	10
# exp.		(3)	(3)	(5)	(5)
Range		(300-321)	(29 - 51)	(188-255)	(4 -24)
Trichloro	4.3	272	37	104	7
# exp.		(5)	(5)	(5)	(5)
Range		(250-296)	(29 - 45)	(1-159)	(5-10)
Phenoxypropionic	1.7	253	24	245	27
# exp.		(4)	(4)	(5)	(5)
Range		(175-328)	(5 - 44)	(215-320)	(22-34)
Dichloro	2.4	342	47	153	20
# exp.		(3)	(3)	(6)	(6)
Range		(297-379)	(2 - 42)	(45-266)	(14-45)
Trichloro	4.5	272	37	114	8
# exp.		(5)	(5)	(5)	(5)
Range		(250-296)	(29 -45)	(82-207)	(5-15)
Phenoxybutyric dichloro	2.5	155	36	11	+45 (2)
# exp.		(8)	(4)	(5)	(5)
Range		(71 -201)	(15-38)	(2-25)	(24-66)

(1) Decrease in phosphate content of reaction media. (2) Increase in phosphate content of reaction media.

The dichloro phenoxypropionic acid, 2(2,4-dichlorophenoxy) propionic acid (dichlorprop) and the trichloro phenoxy compound, 2(2,4,5-trichlorophenoxy) propionic acid (silvex) inhibited the uptake of oxygen about the same extent (55 percent).

The effects of the sodium salts of the phenoxy compounds on oxygen uptake by bluegill liver mitochondria in the presence of alpha-ketoglutarate as substrate are summarized in Table 2. The sodium salts of the phenoxyacetic and phenoxypropionic acid did not alter oxygen uptake by bluegill liver mitochondria. The 2-CPA, 2,4-D and 2,4,5-T inhibited oxygen uptake by bluegill liver mitochondria, and the effects ranged from 58 percent (2,4-D) to 70 percent (2,4,5-T).

Phenoxypropionic acid did not alter oxygen uptake. The dichloro and trichloro substituted phenoxypropionic acid inhibited oxygen uptake 78 and 87 percent respectively.

The effects of the phenoxy compounds on the phosphate uptake by bluegill liver mitochondria are also summarized in Tables 1 and 2. In the presence of succinate, neither phenoxyacetic acid nor the phenoxypropionic acid altered phosphate uptake,

TABLE 2.—Effects of chloro phenoxy compounds on alpha-ketoglutarate oxidase.

		CONTROL		EXPERIMENTAL	
Derivative	g x 10^{-4} per ml Reaction medium	ulO_2/hr/ mg N (Ave.)	umoles PO_4/hr/ mg N (Ave.) (1)	μl O_2/hr/ mg N (Ave.)	μmoles PO_4/hr/ mg N (Ave.) (1)
Phenoxyacetic	1.5	153	32	151	14
# exp.		(4)	(4)	(4)	(4)
Range		(135-169)	(11-72)	(107-171)	(4-35)
2-chloro	1.9	168	40	71	20
# exp.		(4)	(4)	(4)	(4)
Range		(116-214)	(14-57)	(0-153)	(10-48)
4-chloro	3.4	168	40	58	32
# exp.		(4)	(4)	(4)	(4)
Range		(116-214)	(14-57)	(0-88)	(7-48)
Dichloro	3.7	164	22	68	22
# exp.		(4)	(5)	(5)	(5)
Range		(91-214)	(6-33)	(0-131)	(6-33)
Trichloro	2.7	172	40	47	22
# exp.		(3)	(3)	(4)	(4)
Range		(37-229)	(24-49)	(0-119)	(27-42)
Phenoxypropionic	1.7	153	32	156	18
# exp.		(4)	(3)	(4)	(4)
Range		(135-169)	(11-72)	(111-201)	(3-37)
Dichloro	2.4	152	40	32	9
# exp.		(3)	(3)	(3)	(3)
Range		(37-229)	(24-49)	(0-95	(8-15)
Trichloro	2.7	161	52	21	7
# exp.		(5)	(5)	(6)	(6)
Range		(37-229)	(24-91)	(0-56)	(5-19)
Phenoxybutyric dichloro	2.5	131	36	23	+63 (2)
# exp.		(4)	(4)	(4)	(4)
Range		(80-170)	(25-41)	(0-70	(32-81)

(1) Decrease in phosphate content of reaction media.
(2) Increase in phosphate content of reaction media.

but in the presence of alpha-ketoglutarate as substrate, both phenoxyacetic and phenoxypropionic acid appeared to uncouple the uptake of phosphate from the oxidation of substrate. All the chloro substituted phenoxy compounds used in this study did not appear to alter the uptake of phosphate by bluegill liver mitochondria in the presence of either substrate, as th eprimary effects of these chloro substituted phenoxy compounds appeared to be on oxygen metobolism.

The sodium salts of phenoxyacetic acid, 2-CPA,4-CPA,and 2,4,5-T did not alter the hydrolysis of ATP in the presence of manganese, magnesium, or calcium (Hiltibran, unpublished data). The sodium salt of 2,4-D (Hiltibran 1969a) did not alter the hydrolysis of ATP. The sodium salt of phenoxypropionic acid, dichlorprop, and silvex did not alter the hyrolysis of ATP in the presence of manganese, magnesium or calcium (Hiltibran, unpublished data). The sodium salt of 4,(2,4-dichlorophenoxy)butyric acid (2,4-DB) also, did not alter the hydrolysis of ATP in the presence of manganese, magnesium and calcium (Hiltibran, unpublished data).

In the presence of succinate, the chloro group at the 2 position (2-CPA) had a greater influence on oxygen uptake than the chloro group at the 4 position (4-CPA). The presence of a second chloro group at the 4 position appeared to reduce the strong influence of the chloro group at the 2 position, and the presence of a 3rd chloro group at the 5 position appeared to counteract the effect of the chloro group at 4 position and enhanced the effect of chloro at the 2 position, as indicated by the greater inhibition of oxygen uptake in the presence of 2,4,5-T.

In the presence of alpha-ketoglutarate as substrate in inhibition of oxygen uptake did not vary as much in the presence of the various chloro phenoxy compounds, however, the inhibition of oxygen uptake was more severe. This is in contrast to previous reports from this laboratory which have indicated that the succinate oxidase complex of the bluegill liver mitochondria has been affected to a greater extent than the alpha-ketoglutarate oxidase complex in the presence of 2,4-D (Hiltibran 1969b), DDT (Hiltibran 1970a) and metals (Hiltibran 1970b).

The primary purpose was to describe the effects of the chloro groups on the oxygen and phosphate metabolism of bluegill liver mitochondria, but it is possible to pursue further the relation of structure to function on a biological system. Considering the structures of 2,4-D and 2,4,5-T with dichlorprop and silvex, 2,4-D and 2,4,5-T contains an acetic acid analog whereas the dichlorprop and silvex contains propionic acid analog. The data suggests, therefore, the presence of an additional methylene group within the molecule must also exert some influence on the oxygen uptake by bluegill liver mitochondria.

The effect of the acid analog is further illustrated by the more severe inhibition of oxygen uptake in the presence of the sodium salt of 4(2,4-dichlorophenoxy)butyric acid (2,4-DB) in the presence of both substrates, Tables 1 and 2. These data indicate that the acid analog in the phenoxy compounds also has an influence on the oxygen and phosphate uptake by bluegill liver mitochondria. These observations are extended and the biochemical effects of the further modification of the phenoxy compounds by the introduction of esters or salts on the acid analog are discussed (Hiltibran 1972b). Whereas the chloro groups used in this study did not alter phosphate uptake, some chlorophenoxy compounds appear to alter phosphate metabolism (Hilti-

bran 1972b) and some chlorophenoxy compounds also have been found to alter the hydrolysis of ATP by bluegill liver mitochondria (Hiltibran 1972a).

These data extend the observations of Lotlikar, Remmert and Freed (1968) which suggested the position of the chloro group on the phenoxyacetic acid influenced oxidative phosphorylation by cabbage mitochondria (*Brassica oleracea,* var. *capitata* L.).

They reported that compounds with chloro groups at the 4 position inhibited oxygen uptake to a greater extent than the chloro group at the 2 position, and the chloro groups at the 2 and 4 positions were more effective than chloro groups at either the 2 or 4 positions.

In the present study the 2,4,5-T derivatives were more effective on oxygen uptake than were 2,4-D derivatives in the presence of both substrates, whereas silvex was more effective than the dichlorprop in the presence of alpha-ketoglutarate only. These data are in contrast to those presented by Lotlikar et al. (1968). The two trichloro derivatives used in their study were less effective than were the dichloro derivatives. Lotlikar et al. (1968) found that a chlorophenoxy compound containing the butyric acid analog was more effective on oxidative phosphorylation than the similar chlorophenoxy compound containing the acetic acid analog. However, the propionic acid analog was not used in their study. Switzer (1957) reported the 2,4,5-T was slightly more effective on oxidative phosphorylation by isolated soybean mitochondria than was 2,4-D.

These findings indicate a specific interaction of each chlorophenoxy compound with each bluegill liver enzyme complex. There also appears to be a correlation of these effects on oxidative phosphorylation and the toxicity of phenoxy compounds to small bluegills, as the phenoxy compounds which were more toxic to small bluegills than were other phenoxy compounds, altered either oxygen or phosphate uptake by bluegills to a greater extent than phenoxy derivatives, which were less toxic to small bluegills (Hiltibran 1969b, Hiltibran 1972b).

Acknowledgments

The author acknowledges the technical assistance of Charlene Mathis, Karen Mettling, Stephen Forbes, William Maierhofer, Douglas Schemske, Richard Schumacher and Robert Techlaw.

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Manuscript received December 3, 1971.

THE FISHES OF PRAIRIE DU PONT CREEK ST. CLAIR COUNTY, ILLINOIS

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ABSTRACT.—A survey of the fishes of Prairie du Pont Creek, St. Clair county, Illinois, included 41 collections totalling 8,050 specimens of 31 species from 40 sites. The fauna consists of: 1) river and large water species found only in the lower portions of the drainage, 2) ubiquitous small stream species of the area.

Prairie du Pont Creek and its two tributaries Hickman and Sparrow creeks drain approximately 40 square miles in St. Clair county in southwestern Illinois. It is the second major tributary entering the Mississippi River from Illinois below the mouth of the Missouri River, and the first major tributary to enter the Mississippi River below the East St. Louis and Cahokia, Illinois "Metroeast" area. The lower part of the drainage flows from east to west through the Mississippi River bottom lands. The upper parts of the drainage flow south to north on top of the bluff that parallels the Mississippi River on the Illinois side (Figure 1). Hickman Creek and Sparrow Creek flow through the Mississippian limestones and thus usually have rock and gravel bottoms. The upper drainage of Prairie du Pont Creek flows through Pennsylvanian sandstones and shales and usually the bottom is sand and mud. The East Side Levee and Sanitary District of St. Clair and Madison counties has altered the lower portions of Prairie du Pont Creek into a canal system as part of the flood control system for St. Clair county.

The Prairie du Pont Creek system was not studied by Forbes and Richardson (1908) but is of considerable interest as a result of its unique location, the largest deep loess area in the state.

Previous to this study no other known collections have been taken from the Prairie du Pont Creek system (Philip W. Smith, personal communication).

METHODS AND MATERIALS

During this study, 41 collections were made at 40 sites and a total of 8,050 specimens and 31 species were taken. Most of the fish studied were collected between 7 July 1970 to 7 October 1970. All collections are deposited at the Southern Illinois University at Edwardsville campus (SIUE). Collections were made with a 6 by 8 foot woven nylon seine and a 3 by 6 foot woven cotton seine during daylight hours. The collection sites are shown in Figure 1. Localities are given by Putz (1971) dates, collectors, total fishes, and site descriptions.

MINOR SPECIES

A minor species represents less than 1% of the total specimens collected or was found in less than 25% of the collections. The total number of each species collected is followed by sites and respective numbers in each collection. Where a site was collected more than once, the numbers of specimens in each collection are listed in chronological order. Nine species taken only in the lower portions of the drainage are as follows.

Hiodon alosoides (Rafinesque), goldeye; Total-1; A4-1. *Ictiobus bubalus* (Rafinesque), smallmouth buffalo; Total-2; A3-2. *Carpiodes carpio* (Rafinesque), river carpsucker; Total-3; A2-2, A3-1. *Hybognathus argyritus* (Girard), western silvery minnow;

Total-1; A3-1. *H. nuchalis* (Agassiz), central silvery minnow; Total-4; A4-4. *H. placitus (Girard),* plains minnow; Total-1; A2-1. *Notropis blennius* (Girard), river shiner; Total-7; A2-5, A3-2. *Pomoxis annularis* (Rafinesque), white crappie; Total-6; A1-6. *Stizostedion canadense* (Smith), sauger; Total-1; A4-1.

The *H. argyritus* and *H. placitus* were taken from swift flowing water. *H. nuchalis* were taken from pools at site A4.

Eight other minor species are as follows:

Pimephales notatus (Rafinesque), bluntnose minnow; Total-2; E1-1, G1-1. *Carassius auratus* (Linnaeus), goldfish; Total-1; A8-1. *Micropterus salmoides* (Lacepede), largemouth bass; Total-5; A4-1, A7-1, A14-1, A15-1, D1-1. *Lepomis humilis* (Girard), orangespotted sunfish; Total-9; A1-2, A2-2, E7-2, 3. *Ictalurus punctatus* (Rafinesque), channel catfish; Total-1; A18-1. *N. stramineus* (Cope), sand shiner; Total-8; A1-1, A5-2, A6-1, A7-3, C1-1. *N. umbratilis* (Girard), redfin shiner; Total-18; A6-1, A10-1, A12-6, A13-5, A14-5. *Gambusia affinis* (Baird and Girard), mosquitofish; Total-42; A1-33, A2-1, A7-4, B1-3, E2-1.

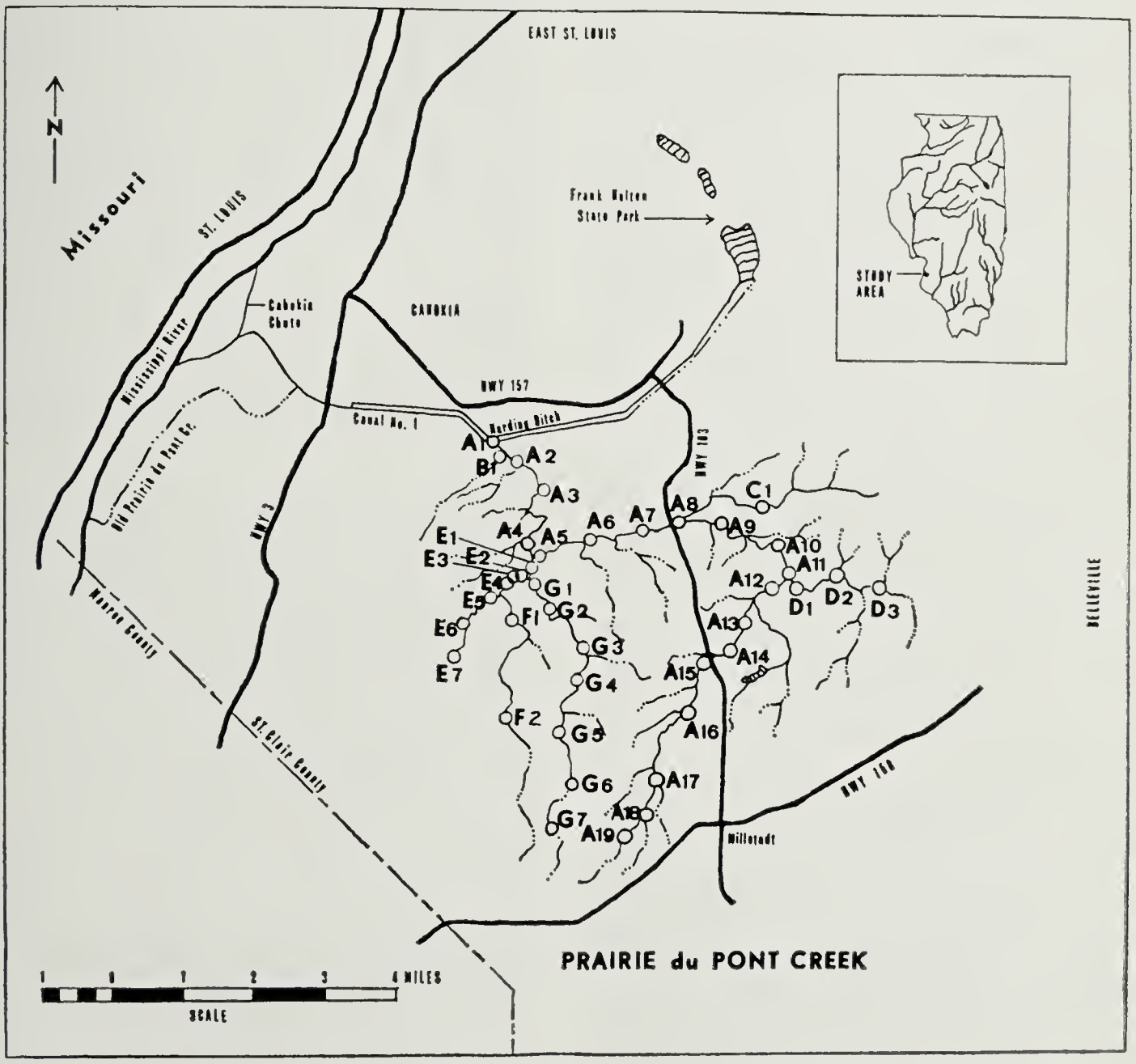

FIGURE 1.—The Prairie du Pont Creek system in South West Illinois. Collection sites marked with open circles. Sites A1-A19 on Prairie du Pont Creek, B1, C1, D1-D3 on unnamed branches of Prairie du Pont Creek, E1-E7 on Sparrow Creek, F1-F2 on unnamed branch of Sparrow Creek, G1-G7 on Hickman Creek.

The *Lepomis humilis* taken at site A1 and A2 were all less than one inch long; however orange-spotted sunfish from just below the spring mouth on Sparrow Creek (E7) were adults.

Major Species

A major species represents at least 1% of the total sample or was found at 25% or more of the collecting sites.

Dorosoma cepedianum (Lesueur), gizzard shad; Total-150; A1-74, A2-34, A3-41, E1-1. The gizzard shad occurs in the lower drainage and was taken mainly from swift flowing water. It made up 1.8% of the total number of specimens collected. The largest gizzard shad taken was 10¼ inches long, but most of the others were under 4 inches in length.

Catostomus commersoni (Lacepede), white sucker; Total-46; A4-1, A8-1, A11-1, A15-4, A16-1, A17-1, A18-5, E1-4, E2-3, E3-7, E4-3, E5-5, E7-2,1, G1-6, G6-1. The white sucker is the common sucker in the drainage and was found in 37.5% of all collections. It was scattered throughout the drainage in small numbers mainly in the deeper pools, but was most abundant in spring fed Sparrow Creek. White suckers up to 14 inches long were taken from Sparrow Creek. These are the first known collections of *Catostomus commersoni* from St. Clair county (Philip W. Smith, personal communication).

Campostoma anomalum (Agassiz), central stoneroller; Total-930; A4-4, A6-1, A8-4, A9-20, A10-17, A11-36, A12-10, A13-16, A14-92, A15-51, A16-29, A17-7, A18-105, A19-20, B1-6, C1-1, D1-28, D2-16, D3-16, E1-34, E2-25, E3-14, E4-38, E5-4, E6-4, E7-2,9, G1-56, G2-63, G3-64, G4-23, G5-60, G6-51, G7-4. The stoneroller is the third most abundant species in the drainage, 11.5% of the total sample, and was collected at all but 7 sites.

Cyprinus carpio (Linnaeus), carp; Total-61; A1-18, A2-16, A3-8, A4-6, A5-1, A8-4, A9-2, A10-1, A11-1, A14-1, A15-1, E1-1, E2-1. Carp were most abundant in larger pools in the lower drainage and were found at 32.5% of the collection sites. The carp taken at A1, A2, and A3 were all under 3 inches in length but small carp were not taken in the upper part of the drainage. Carp taken above A3 ranged from ¼ lb. to 1 lb.

Notemigonus crysoleucas (Mitchill), golden shiner; Total-152; A1-3, A3-1, A5-3, A6-6, A7-9, A9-21, A10-6, A11-9, A12-21, A13-8, A14-1, C1-32, D1-22, D2-7, D3-1, E1-2. The golden shiner was most abundant in the main branch of Prairie du Pont Creek. Our data supports Trautman (1957) "small populations occurred in weedless waters which frequently were silt laden and had a clayey silt bottom; but only strays or relict populations occurred in habitually silt laden waters, and especially where silt deposition on the botton was rapid." The golden shiner made up 1.8% of the total number of fish collected and was found at 40% of the collection sites.

Notropis atherinoides (Rafinesque), emerald shiner; Total-192; A1-94, A2-69, A3-19, A4-10. The emerald shiner was collected only in the lower portions of the drainage. This agrees with Smith (1965) who lists it as occurring in the larger and medium sized rivers throughout Illinois. The emerald shiner made up 2.3% of the total number of specimens collected.

Notropis dorsalis (Agassiz), bigmouth shiner; Total-1,779; A4-28, A5-5, A6-1, A7-69, A8-109, A9-41, A10-17, A11-7, A12-2, A13-31, A14-94, A15-52, A16-60, A17-20, C1-151, D1-37, D2-30, D3-21, E1-171, E2-4, G1-312, G2-197, G3-318, G4-2. The bigmouth shiner made up 22.2% of the total

sample and was the second most abundant species. It was found at 60% of the collection sites but was most numerous where the bottom was mostly sand.

Notropis lutrensis (Baird and Girard), red shiner; Total-432; A1-35 A2-56, A3-88, A5-18, A6-1, A7-2, A8-7, A10-21, A11-8, A12-18, A13-96, A14-23, A15-11, A16-4, A19-3, B1-4, D1-20, E1-14, E2-2, E4-1. The red shiner was found at 50% of the collection sites and made up 5.3% of the total sample. It was the fifth most abundant species in the drainage. The red shiner was more common in the lower drainage.

Pimephales promelas (Rafinesque), fathead minnow; Total-28; A4-2, A8-4, A16-1, A17-5, A18-4, A19-4, C1-1, E1-1, E2-1, E4-1, G1-1, G2-1, G4-2, The fathead minnow was found in small numbers throughout the drainage over mud bottom at 35% of the collection sites.

Semotilus atromaculatus (Mitchill), creek chub; Total-3,073; A4-155, A5-10, A6-6, A7-7, A8-61, A9-64, A10-94, A11-29, A12-33, A13-31, A14-101, A15-62, A16-41, A17-60, A18-186, A19-85, B1-42, C1-17, D1-77, D2-62, D3-35, E1-241, E2-119, E3-26, E4-200, E5-14, E6-48, E7-4,8, F1-16, F2-159, G1-180, G2-151, G3-186, G4-15, G5-167, G6-214, G7-47. The creek chub occurred at all but three sites and was the most abundant species, (38.1% of the total sample). A 9 inch specimen was collected at site D1.

Ictalurus melas (Rafinesque), black bullhead; Total-101: A1-2, A2-1, A4-2, A9-1, A11-23, A12-6, A13-1, A14-1, A15-10, A16-1, A17-11, A18-7, C1-3, D1-1, D2-1, D3-16, E7-6,5, F1-1, F2-5, G1-1, G2-2, G5-2, G6-2. The black bullhead was taken mainly from the deep mud-bottomed pools throughout the drainage. It was sometimes kicked from underneath tree roots or undercut banks. The black bullhead represented 1.2% of the total sample and was found at 46% of the collection sites.

Lepomis cyanellus (Rafinesque), green sunfish; Total 191: A2-3, A3-2, A4-3, A5-6, A6-7, A8-5, A10-2, A11-1, A12-1, A13-11, A14-12, A15-7, A16-2, A17-4, A18-29, A19-8, B1-3, D1-2, D2-2, D3-3, E1-3, E2-2, E2-4, E4-1, E5-2, E6-4, E7-16,20, G1-5, G2-2, G5-1, G6-18. The green sunfish is widespread in the system. It was found in 77.5% of the collections and made up 2.3% of the total sample.

Lepomis macrochirus (Rafinesque), bluegill; Total-172; A1-88, A2-68, A3-2, A4-1, A6-1, A10-1, A17-2, B1-1, E1-2, E2-2, E3-1, E5-1, E7-1, G2-1. Blue gills are scattered throughout the drainage in small numbers. Small specimens were abundant at sites A1 and A2. It was found in 35% of the collections and made up 2.1% of the total sample.

Etheostoma spectabile (Agassiz), orangethroat darter; Total-631; A4-1, A9-1, A11-10, A12-1, A14-8, A15-28, A16-8, A17-10, A18-69, A19-3, B1-5, D1-5, D3-12, E1-142, E2-64, E3-27, E4-6, E5-6, E6-8, E7-5, G1-68, G2-37, G3-61, G4-14, G5-39, G6-3. The orangethroat was abundant in the upper parts of the system. It was collected mainly from the riffles although many specimens were taken from shallow pools. The orangethroat was the fourth most common species and made up 7.8% of the total sample and was found in 65% of the collections. Large orangethroats up to 2.6 inches long were taken from site E6 on spring fed Sparrow Creek. Large population of orangethroat darters was present at E6 but the large rocks in the riffles prevented effective collecting.

Discussion

The lower portion of Prairie du Pont Creek at least upstream to A3

is a highly unstable habitat under the influence of the Mississippi River and has certainly been greatly altered by the canalization of the lower part of the creek. Before canalization the lower part of Prairie du Pont Creek was probably alternating riffles and deep pools such as seen at site A4. Now the lower part of Prairie du Pont Creek during low water is from 1 foot to 3.5 ft. deep with the water moving swiftly along over a thin layer of sand with soft mud underneath. Water now backs up into the creek system when the Mississippi River is high. The water is sometimes 15 to 20 ft. deep for a week or longer. Before canalization the river probably did not flood the lower part of Prairie du Pont Creek as it does now. When the river is up, many river fish migrate up into the lower portion of Prairie du Pont Creek. Local residents put out hoop nets and have caught buffalo and carp up to 30 lbs. during high water. In the fall of 1970 Frank Putz saw *Lepisosteus platostomus, Lepisosteus osseus and Aplodinotus grunniens* caught from the lower portion of the creek system (around A2) during high water.

Sparrow Creek originates from a spring approximately 12 feet wide which flows slowly from the base of a limestone bluff. Sparrow Creek is a clear cold stream. At site E4, Sparrow Creek makes a series of small water falls as it flows down a Mississippian limestone bluff.

Acknowledgments

We wish to thank the persons who helped collect: George Defrates, Ed Domyan, T. C. Henderson, Richard Cygan, Russell Maloney, and especially Michael Putz. Dr. Philip W. Smith and Larry Page of the Illinois Natural History Survey aided with the identification of fishes. Dr. Dorothy Gore, Southern Illinois University at Edwardsville, supplied the information on the geology of the region. Dr. Leslie J. Wehling offered helpful criticisms of the manuscript. We also wish to thank Patricia Bauer who typed the manuscript; Janet Mulqueeny and Peggy Coughlin for help in proofreading the manuscript.

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Manuscript received January 10, 1972.

SYMPOSIUM, 1971
PRE-COLLEGE SCIENCE EDUCATION — SPECIAL EMPHASIS ON SCIENCE IN ELEMENTARY GRADES

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In recent years, we have heard of the success of new science programs in all levels of our public schools. Most notable are the innovations in science programs for the elementary grades designed to prepare students for their future; to cope with more science and technology in the everyday events of their lives and to prepare them for constant scientific and technological changes. The science teachers must be trained in a manner that leads to their success with these programs. The trend to more lectures and less laboratory experience in introductory college and university science courses must be reversed, since elementary teachers and others majoring in non-science areas will not be able to understand the programs based on doing science without themselves learning science skills and developing understandings of science and technology. They must have and create in their students a positive attitude about science and technology and instill a long-lasting interest in both.

Today we are fortunate to have three speakers to discuss some of the issues in pre-college science education, an area of long interest to this Academy. This symposium was made possible by an award to the Illinois State Academy of Science from Bradley University, the host of this 64th Annual Meeting. It is with great pleasure that I present to you: Dr. James Joseph Gallagher, Director of Academic Development, Governors State University, Park Forest South, Illinois; Dr. Paul DeHart Hurd, Professor of Education, Stanford University, Stanford, California; and, Dr. Leopold E. Klopfer, Director, Individualized Science, Learning Research and Development Center, University of Pittsburgh, Pittsburgh, Pennsylvania.

AN OVERVIEW OF THE SYMPOSIUM ON PRE-COLLEGE SCIENCE EDUCATION

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In keeping with the title of my presentation, I shall try to provide a historical context for the presentations of the two main speakers. In so doing, I will try to show in a manner of minutes where elementary science has come from, where it is now, and where it might be headed in the future.

As with most other educational enterprises, the teaching of elementary science is the victim of fads. During the past 150 years, two broad themes have alternated in popularity in elementary science teaching in this country. One of these, an attitudinal theme, has placed heavy emphasis on developing positive attitudes and values regarding the natural world. Examples of programs in which the attitudinal theme has dominated are

the science books written for children during the early part of the 19th Century and the Nature Study Movement of the early 20th Century.

The second theme which has alternated in popularity with the attitudinal theme stressed the disciplines of science. In programs influenced by this disciplinary theme, children learned about many of the major conclusions of science, and their applications in technology. Instruction was largely content centered. Examples of this alternative include many of the textbook programs of the 1940's and 1950's and science programs developed in the 1880's — perhaps one of the most outstanding of these was developed by E. G. Howe at the training school in Champaign.

In the 1960's, elementary science teaching was given a new direction. More than a dozen federally supported projects were developed during that decade. Although these modern elementary school science projects vary in structure and philosophy, they have two common features. First, they are based on an expanded conception of children's learning; and second, they represent a new and deeper understanding of the nature of science as it is known to scientists.

The expanded conception of children's learning grew out of the work of psychologists such as Robert Gagné, Jerome Bruner, and Jean Piaget, and has resulted in the design of instructional programs which provide children with first-hand experiences as a basis for learning. A view of classrooms in which modern elementary science programs are being taught will show small groups of children actively involved in doing "experiments," with the teacher serving as a guide to inquiry, instead of a purveyor of information.

The new, deeper understanding of the nature of science, which is represented in these programs, arose from the involvement of top level university scientists in the design of instruction for elementary school children, and has resulted in a new form of content for elementary school science. This content stresses the intellectual techniques or processes of inquiry which scientists use to acquire and interpret information about the natural world. Thus, when children learn from these new programs in elementary science, they make observations, raise questions, plan experiments to answer them, gather data, and interpret data in ways similar to those used by practicing scientists. In contrasting the content of traditional elementary science programs with that of modern ones, it can be said that the traditional programs emphasized *what* is known, whereas the modern ones stress *how* it is known.

Professor Hurd, in his presentation, will describe in greater detail the modern programs and the philosophy that underlies them.

In spite of the many great strengths of the modern programs some difficulties have arisen in their use. I will outline three of these in rapid succession: First, implementation of the modern elementary science programs demands a new role for teachers; and by-and-large, teachers have not been able to adjust to it. The new role requires that teachers guide students in raising questions and finding their own answers. Difficulties in implementation arise because teachers, generally, are "tellers," not "guides." Second, in the modern programs, individual differences among students are accentuated. Children learn difficult things from their experiences and they progress at different rates. It becomes difficult for teachers who are used to having children all learn the same content and progress at relatively more uniform rates to adjust to the increased disparity in progress.

In his talk, Professor Klopfer will describe an approach to resolving these two problems thru individualizing instruction. The third problem relates to children's reactions to the content of the modern programs. For many children, this content lacks relevance. It is too abstract in its relationship to the real world that exists outside of school. I agree with the scientists who helped to formulate these programs that the content represents valid science. However, it is valid science in our adult eyes, but children do not see it as being significant.

To resolve this problem of lack of relevance, I suggest that elementary science teaching for the 1970's must focus on issues and problems that children can grasp and deal with. In more specific terms, the environment in which children live provides a focal point, a context for elementary science teaching and learning. The environment which children know is comprised of both natural and man-made phenomena. The environment provides a context for unifying the two themes that I spoke of earlier, (i.e., the attitudinal theme and the disciplinary theme) which alternately have influenced elementary science teaching for the past 150 years. The environment also provides a context for unifying the disciplines of science and a way of interrelating school experiences and those on the outside, making them relevant to one another. Moreover, utilizing children's natural and man-made environments provides a setting in which knowledge of subject matter can be developed along with attitudes and values necessary for intelligent participation in our complex, changing society.

I am not suggesting that we abandon the advances in elementary science teaching that were made during the past decade. Nor am I suggesting that we re-introduce nature study, or conservation education, or outdoor education in place of elementary science. Rather, I am suggesting that we integrate the attitudinal and disciplinary themes while building on the recent instructional advances. We can integrate themes and build on these advances if our elementary science teaching helps children develop inquiry skills, as in the modern elementary science programs, and, in addition, if it helps them to learn how to use these skills to understand their natural and man-made environment and resolve its problems. We can integrate the two themes and we can build on these advances if our elementary science teaching helps children become involved in first-hand experiences as part of the learning processes, and if these experiences are related directly to the environment. We can integrate the two themes and we can build on these advances if our elementary science teaching provides opportunities for children to do experiments, and if these experiments deal with questions which children raise about the environment. We can integrate the two themes and we can continue to build on these advances if our elementary science teaching includes valid processes and principles of sciences as a basic part of instructional programs, and if these principles and processes represent to children the ways that scientists, and all thinking people, approach the solution of practical problems about the natural and man-made environment.

Integration of the attitudinal and disciplinary themes in ways that build on the educational advances of the modern elementary science programs will not be without its own difficulties. Popular concern over our natural environment, coupled with difficulties in implementing m o d e r n elementary science programs, may result in another swing of the pendulum of em-

phasis in elementary science teaching back to the attitudinal theme which characterized Nature Study. This would be unfortunate since elementary science in this decade should not be dominated by one theme or the other. Instead, we should seek integration of the attitudinal and disciplinary emphases while capitalizing on the best of contemporary knowledge about the nature of science and the learning processes.

MODERN ELEMENTARY SCHOOL SCIENCE

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Early in the 1950's it became apparent that the precollege science curriculum in the United States was essentially meaningless for understanding modern science. The subject matter was frequently outdated, often as much as a half century, and courses were overloaded with trivial information of little importance for comprehending today's science. Furthermore, in an attempt to provide 'coverage' of a field, the textbook treatment of a topic became shallower as the complexity of the ideas involved increased. Courses in science were overloaded with factual information and devoid of basic concepts and theories.

Late in the 1950's a major effort, funded by the National Science Foundation, was initiated to reform the teaching of science, first in secondary schools and later in the elementary schools. The task, placed in the hands of research scientists, included the directive "to improve course content." After examining the subject matter of typical courses in wide use at the time, the separate curriculum committees decided to develop new programs in biology, chemistry, physics and earth science. Each curriculum project operated under the assumption that the new courses should represent a more rigorous treatment of science, displayed in the manner as it is known to research scientists. Thus, the student would obtain a valid picture of the *science* of biology, chemistry or physics. To know the theoretical, investigative, and conceptual basis of a particular discipline became the primary goals of the new science.

To achieve the new goals of science teaching, a few conceptual ideas, central for understanding the structure of a discipline, became the core of each course. Through an in-depth exploration of these concepts young people would acquire a notion of what scientific thought means. The laboratory experiments designed for the new courses consisted of 'open-ended' investigations which afforded practice in scientific inquiry.

Keeping the new courses faithful to the disciplines of science meant omitting the majority of technological and applied topics found in traditional science textbooks. Here again, the assumption was made that science taught in the way a scientist understands it 'would make it inherently interesting' for the non-scientist and technological considerations would only serve to confuse the meaning of science. All too briefly, this represents the general tone of secondary school science improvement efforts over the past dozen years. A detailed description of this period has been presented elsewhere (Hurd, 1969.)

The science curriculum reform movement of the 1960's brought forth a number of innovative ideas, for example, improvements in curriculum design and innovative instructional materials. The importance of teaching style in relation to instructional ob-

jectives gained recognition as did the need for students to have a greater responsibility in thinking through science oriented problems on their own. But for all these gains and others, the effectiveness of the new courses in terms of increasing student interest in science and developing an appreciation of the scientific enterprise as it relates to the intellectual and material progress of mankind has been disappointing, if not negative. Decreasing enrollments in science courses in high school and college, as well as the current distrust of scientists, are illustrative of the unfavorable impact.

The social and cultural conditions which gave rise to the science curriculum improvement projects of the 1960's are *not* the conditions of 1970. The emerging problems that concern both scientists and citizens suggest that again we need to reexamine the goals of science teaching. Science has now become broadly integrated into all phases of our culture. A close partnership has developed between science and the economic, social, and political issues of our time. The scientific enterprise is also under pressure; there is a strong ferment to make the scientific researcher more socially responsible. Technologists and engineers are being forced into the position of assessing the potential impact of technological developments b e f o r e widespread introduction into the economy. These pressures represent the present concerns of the common man who somehow feels science and technology have little concern for him as a human being. Ironically, he sees little connection between scientific achievements and the possibilities for personal, cultural and social betterment. He does not recognize the potential science holds for the whole of life and how it can affect the future of man; nor does he appreciate the history of modern science and its influence upon American civilization from the beginning. Most of all, the average citizen does not know what science is, nor does he understand the requirements for effective living in a modern scientific-technological-industrial society. It is in each of these ways that the science teaching of the 1960's has fallen short of an appropriate education in science for the non-scientist.

Science Education for the 1970's

The broad goal of science teaching for the 1970's needs to go beyond the restrictive context of the special disciplines and consider science in relation to the affairs of mankind, the actualities of the 'real' world, and the human condition. The scientific enterprise becomes meaningful for the common man only in a cultural and social context. The task of general education is to make it possible for the student to bridge the gap between *knowledge in being* and *knowledge in action.* Knowledge lacks significance at any time unless it can be applied to problems or used to seek new goals. The scientific researcher applies his knowledge to advance the frontier of his discipline; the nonspecialist wishes to employ science information to cope with personal and social problems in a way that will make a difference in human living.

Looking back to the 1960's we find considerable emphasis in all the new curricula on developing skills of scientific inquiry. Students are engaged with problems in the laboratory and field which will presumably require them "to think like a scientist and to use the strategies of scientific investigation." However, the p r o b l e m s which students face are those of life; they are 'real,' they are complex and not subject to simplification or to the precise controls of a laboratory experiment. Out of class the student must face science-social questions, and answers to these always involve

a consideration of people. Any satisfactory solution depends upon treating data in a *qualitative* fashion with a consideration of human values. The problem-solving process is essentially one of decision making. Whatever answer is formed depends as much on politics, consensus, trade-offs, personal choice, group dynamics, follow through, feedback, and the weighing of advantages as it does on reliable information. The worthiness of an answer relies more on the contribution it makes to significant social action than on whether it is consistent with a relevant theory of science. This does not mean the student behaves in an irresponsible fashion; we expect his thinking to be disciplined and rational, using knowledge from science for making as reliable judgments as possible.

Social problems based in science are seldom confined to a single discipline but have overlaps, not only within science but with other fields of human endeavor which may include the arts, philosophy, religion and the behavioral sciences. The need is for a greater inter-penetration of subject matter within the sciences and the establishment of 'bridges' between the sciences and other fields of learning. This is especially important if we expect the student to become a better citizen in the sense of being more informed, more concerned, and more competent to reach science-social decisions.

It is not being suggested that the virtues, processes and concepts of science be taught with lesser meaning but that they be taught in a societal context and in relationship to other disciplines. A science based culture cannot be understood by the separate study of disciplines, each focusing on its own body of technical information and autonomous theories. The science knowledge of most worth for general education is that which reflects the spirit of science, the social, the cultural, the technological and the humane phases of the scientific enterprise. The educational irony of this century has been to acquaint young people with the great ideas and achievements of science and then to deny them any insights into the social arrangements which can make these achievements of recognizable benefit to mankind.

It is quite proper that young people get a conceptually coherent presentation of a scientific discipline in a course; but if they fail to become acquainted with the interplay of issues and controversies that led to this knowledge, then they have been indoctrinated. And if they have not been given an insight into how this knowledge contributes to the intellectual culture of our times and to the resolution of social and technological problems, they have spent their class hours on unrealistic activities. This is where the thinking about science teaching for the non-scientist now stands and suggests the curriculum context for the 1970's.

Science in the Elementary School

Science in one form or another has always had a place in the elementary school curriculum. This is not to suggest, however, that a scientist would recognize the subject if he observed a class in session. The point is best illustrated by a brief history of science teaching in American schools.

From *1700-1850,* elementary science teaching consisted of having children learn the names of rocks, plants, animals, planets, constellations, and cloud formations. The major educational goal was to have children acquire a fund of *interesting information.*

Sometime around the year 1850, and continuing to about 1890, science was taught for religious purposes and for the development of morals. One typical science lesson of this period

was introduced as follows: "Knowledge and virtue are like the rays of light and should act upon the heart in a very similar manner." There were many stories about the "bad old wolf," "the wise old owl," and the "sly old fox." The major goal for the teaching of science in this period was to instill *moral values* in children.

From 1890 to 1900, we find the rise of "faculty psychology." The essence of this educational movement was: "Mental f a c u l t i e s grow and are strengthened by exercise and we pursue certain studies not because we think we shall be called upon to apply them, but because we know they make the mind fitter . . ." Under the stimulus of faculty psychology, elementary science was taught in order to "exercise" the child's memory and to sharpen his powers of observa tions. To these ends much of the science teaching consisted of collecting objects, making classifications, and learning the technical name of things. The primary reason for teaching science at this time was to *strengthen the mind* of the child.

At the turn of this century, the American scene was changing rapidly. The closing of the frontier and the rapid growth of industry led people to migrate from rural areas to cities where they could be employed. This was the beginning of the great industrial age in American history and a new era in science. One might expect that the impact of these events would generate a new approach to science teaching in the elementary schools. It did, but in an unexpected way. The innovative science curriculum of the time was *nature study,* a movement that was to last well into the 1920's. The program was developed by members of the science faculty at Cornell University. Its primary purpose was to generate a love and interest in living things, especially those found in rural areas of the country. Perhaps if there was a greater feeling for nature, fewer people would migrate from farms to the city. Nature study differed from earlier elementary science programs in that it was a carefully organized curriculum and had a meaningful sequence. Whatever hopes the designers had about teaching children science through nature study were lost in the ensuing years. A few examples of the objectives will illustrate what happened to the "science" of the program: "To keep alive the use of poetry"; "To cause the child to appreciate plants and animals for no other reason aside from loving them." Pictures were painted of plants and animals and hung about the classroom, songs sung, and poetry written. The major goal of the nature study movement was to instill a *love of living things.* The great developments in science and technology going on at this time had little effect on the teaching of elementary school science.

In 1927, largely through the efforts of Gerald Craig, then a doctoral student at Teachers College, Columbia University, a new concept of science teaching for children was developed. In his program, children had an opportunity to learn about airplanes, automobiles, and trains through a study of transportation. Work on communication consisted of knowing how such inventions as the telegraph, telephone and radio operated. The course of study had as its primary goal the *social use of science through technology.*

Elementary science textbooks in the period from 1940 to 1950 were referred to as "science readers." Children's interest in science made it a good vehicle for *the teaching of reading,* which at this time was the major reason for teaching science.

Educators for some time have felt that children learn best when they are actively involved with the mater-

ials of learning. Around 1950 the "activity movement" in education began to influence the teaching of science. It took the form of having children do "experiments." Children found that magnets pick up some materials but not others. Doorbells were modified to make a telegraph receiving instrument. Air pressure was demonstrated by collapsing oil cans, an experiment repeated in nearly every grade level, and while the can was usually larger in the upper grades, the underlying science concept did not increase in meaning. The teaching of science at this time was something like a magician show — cans collapsed mysteriously and child-made volcanoes belched smoke and fumes. The major goal for teaching science now became *to provide children with activities,* mostly for the sake of the activity.

By 1960, the science teaching reform movement going on in the secondary schools led to an examination of science teaching in the elementary schools. Scientists were amazed by what they found; there was little in these programs they could recognize as being science. It was apparent that something needed to be done to bring the "science" teaching activities of the elementary school more in line with the way science is. To begin this reform was not easy, mostly because no one, excepting the scientist, was complaining. Teachers, at least those who taught any science, liked what they were doing because children liked to make volcanoes, had fun playing with magnets, and enjoyed seeing cans collapse for no apparent reason. Parents were not unhappy with the program because their children could tell them what happened in the science class today; their children were not so sure about what happened in other subjects. Who was unhappy then? — the scientist. He saw children having an interesting time playing with various materials but with *no educational pay-off* to the child. What's more, much of the teaching was inaccurate as science and would need to be retaught at a later time to correct the error, usually not a successful effort.

The Elementary School Science of the 1970's

In 1962, a nationwide Commission on Science Instruction (AAAS. Commission on Science Instruction. 1515 Massachusetts Avenue, N.W., Washington, D.C. 20005) was established to stimulate the development of innovative approaches for teaching children science. Nearly a dozen curriculum projects have been initiated since this time, each with a special point of view, but all with the idea of presenting science in its "true" character and with educational goals that are suited to modern times. The details of these programs are presented elsewhere (EPIE, 1970; Hurd and Gallagher, 1968).

These science programs have had the advantage of extensive testing in classrooms under a variety of learning conditions. The task has not been an easy one for the curriculum designers for several reasons:

1. It was immediately evident that not much is known about how children learn or what science they are able to learn at different maturity levels. Although children are of the same species as adults, they do not learn in the same way or at the same rate. A number of psychologists assisted in furnishing data on children's learning and the meaning it may hold for curriculum development in elementary science.

J. Bruner — provided ideas about learning readiness and the process of education.

R. Gagné — provided a model for the hierarchial development of intellectual skills and the notion of a cumulative curriculum.

J. Piaget — provided data on the developmental levels of children and how these relate to the formation of certain science concepts.

2. A second problem was to find science subject matter that would have a long tenure of intellectual usefulness and be generalizable, such as ideas about systems, time-ordered events, growth of a population, symmetry, and interaction.
3. Another problem was to devise the "instructional mode" or "teaching style" to assure the success of the new science programs. Finding a teaching style, that complements the investigative process of science, has always been a problem in science instruction. The importance of doing so was recognized as early as 1884 by Charles Eliot, a chemist and longtime president of Harvard University; he wrote as follows: "There is very little profit in studying natural science in a book, as if it were grammer or history; for nothing of the peculiar discipline which the proper study of science supplies can be obtained in that way, although some information on scientific subjects may be so acquired." In the present curriculum reform the appropriate style of teaching is described as an "inquiry," "discovery," or "process" approach (actually there are some differences between each of these approaches, but the intent is similar).
4. Then there was the problem of developing instructional resources consistent with the way science should be learned and which would likely assure the desired learning. This meant the development of learning materials which would engage children in an exploratory manner where information is acquired through direct experience. There are no textbooks in the new elementary school science.

Teaching the Elementary Science of the 1970's

How does one go about teaching children to help them form concepts? We should be clear on the point that one does *not* really teach a child a concept, but rather provides favorable conditions and materials which have the potential for stimulating the child's insight into a set of circumstances from which a concept may emerge. For example, we want a first grade child to develop a notion about what measurement really means. We begin by asking several children to throw bean bags toward a line on the classroom floor. Then the children are asked how they can tell whose bean bag got closest to the line. Someone suggests that they could measure the distance from the line. Now the teacher provides each child with a stick so they can begin measuring. It takes only a minute of waiting and we find the children all excited because things are not going right. What is wrong? They have noticed the sticks are all of different lengths. The teacher at this point simply says, "What can we do?" One child says, "It is only fair if everybody has a stick of the same size." The class selects one stick by popular vote, gives it a name like "XPOO," and begins to find out who threw the bean bag closest to the line by counting the number of XPOO's. Now let's examine what these first grade children have learned, and all on their own. They have, through direct experience, come to the conclusion

that if one is to measure distance it is only fair if everyone uses the same unit of measurement — a standard unit.

Tomorrow the same procedure with the bean bags is repeated but this time on the playground and with the instruction to throw the bags as far as possible. The children are now asked to find out who threw his bean bag the farthest. Again there is a turmoil, this time because the children find they cannot count high enough to use XPOO's for measuring the distance to the farthest bean bag. The teacher's only comment is, "What can we do about this?" One child remarks, "If we had longer sticks we could count high enough to find out who is the best bean bag thrower." The teacher supplies longer sticks, the children select one and give it a name, such as "big poo." What have the children learned today that contributes to their developing concept of measurement? They have learned through their own efforts again that a unit of measurement works better if it is appropriate to the distance to be measured, just as adults have found it inconvenient using inches to measure the distance to town and so prefer miles, which astronomers find intolerable for their work. With 10-15 more "measuring" experiences of various kinds, such as judging temperatures, weighing, and determining speed, the child begins to perceive what measurement is all about; in other words, he forms a concept of measurement. This kind of teaching is much different from having children learn how many miles it is to the moon, sun, and planets, and hoping they will acquire a concept of distance, which they will not.

The Teacher for the New Elementary School Science

She is not a teller of tales; she listens, observes and gently guides by questioning. She does not steal the joy of learning from children by giving away answers. She helps children inquire into nature and phenomena, to work on their own, and strives to wean them from dependence on herself and a textbook. She helps children form ideas. She provides time for a child to internalize what he is trying to learn. She seeks to teach in 5 days what she used to tell in 5 minutes. And she believes that as long as a child needs a teacher he is not yet educated.

The Pupil in the New Elementary Science Class

He is the center of activity, developing an inquiring mind, building confidence in his ability to learn on his own. He also soon discovers he can have a divergent idea without being considered either a "smart-alec" or a delinquent. He learns what it means to fail; progress in his class is based on successful achievement. What's more, he builds a feeling for the "power of knowing," that knowledge once acquired can be used to discover new relationships and to generate new ideas.

The Organization of the New Elementary Science Programs

The new programs are not all alike; they utilize different subject matter and have unique patterns of curriculum organizations.

—Some are organized around the major ordering concepts of science (systems, interactions, life cycles): Science Curriculum Improvement Study (SCIS) and the Conceptually Oriented Program in Science (COPES).

—Some focus on the sequential building of intellectual processes (measuring, observing, classifying): AAAS, Science — A Process Approach.

—Some stress an open curriculum, seeking to preserve the child's natural exploratory curiosity through the study of conceptually rich instructional materials (behavior of meal worms, batteries and bulbs, microgardening): Elementary Science Study (EES).

—One program combines arithmetic and science, using each to sustain the learning of the other: the Minnesota Mathematics and Science Teaching Project (MINNEMAST).

—Other programs place more stress on a particular style of teaching: the Individually Prescribed Instruction Program in Science (IPI Science).

Although there are differences between each project, all programs seek to develop an inquiring mind; to maximize the learning potential of the child; to build intellectual competence; to develop a self-sustaining learner; and to increase the perceptual awareness of children. They all seek to assure a greater transfer value to what is learned through teaching concepts and inquiry processes of wide generalizability, also a device for improving the retention of what is learned.

The New Elementary Science and Educationally Disadvantaged Children

The use of the new programs with disadvantaged children has produced surprising good learning results. Because science has a base in real objects and things which can be manipulated, children do not need an initial backlog of verbal symbols to study science. A weakness of the disadvantaged learner is that frequently he does not have an adequate vocabulary or it does not meet standard curriculum demands. In using the "new" elementary science materials one finds less difference in performance between the advantaged and disadvantaged. The new science is not abstract, the child is always working with real things, he is doing something — comparing, counting, bouncing balls, or just plain wondering. When he needs a word symbol he has had a direct experience which calls for a way to express it. Because it is easy for children to ask questions about science phenomena, their linguistic skills are improved. The learning of science does not have to be formalized. There is a naturalistic approach in the earlier phases, an interplay of observations and interpretations in terms of the real world, and this is where motivation for learning arises. The child's freedom to explore, manipulate, speculate, and wonder provides the essential stimulus to learn.

In an abbreviated way this is the "picture" of the new elementary science for the 70's, and an exciting time it can be for children. What are the major purposes for teaching modern elementary school science? They are to help the child *grow up intellectually* and to develop *a sustaining interest* in the natural environment.

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INDIVIDUALIZED SCIENCE IN FOCUS

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Individualized Science is an elementary-school science education program that is relevant. It is relevant to the child and it is relevant to the social circumstances of the day. The program has been under development since 1964 at the Learning Research and Development Center, University of Pittsburgh, in cooperation with the Oakleaf School, a public elementary school in the Baldwin-Whitehall School District. In this paper, I would like to discuss the concerns that underlie the design of *Individualized Science* and to describe the main features of the program's organization and operation which represent our response to these considerations.

In designing a school science program, we must think first about the child. This does not suggest a maudlin, hand-wringing concern about the child, but a realistic appraisal of the child's need for orientation in the world. We know that the world which the child faces daily shows itself to him as a buzzing, booming confusion filled with contradictions. How can the study of science help the child to unravel some of these contradictions and to make some sense out of his world? The young child in the elementary school, if he is thoughtful, —and what child is not thoughtful in his own way? — is probably most concerned with the functions of his own body and with what transpires in his immediate surroundings. It doesn't seem unreasonable, then, that the child's experiences early in his science program should try to help him to unpuzzle things in those areas. The child has many other pushes and tugs, — differing curiosities, questions, interests. These vary widely from one child to another due to differences in background, capacities, and as-yet-unfathomed aspects of personality. It is therefore apparent that the study of science should seek to give each child some methods and skills for attacking the questions which tug at him and some useful frameworks in which to fit the answers. Perhaps what an elementary-school science program really can accomplish is to make the child's world appear less contradictory to him, so he may feel safe in it.

Our second concern in designing a science program is that the subject-matter content should be relevant to the social circumstances of today and the foreseeable future. All of us are aware of the problems and issues raised by the unbridled growth of human population, the pollution of our natural environment, the everyday hazards of living in a technological age. To understand and seek solutions to these and other problems of contemporary society, the individual should have a firm grasp of the essential scientific ideas related to these problems. Since the life of the elementary-school student is and will be mightily affected by how these problems are resolved, and he may even be a participant in solving them, what better place is there for him to begin to develop understanding of the relevant scientific ideas than in his study of science? Five groups of current and imminent social problems contain a sizeable science component, a subject-matter pool from which the science content in most of the instructional units of *Individualized Science* is drawn. The units are organized around the science content, not around the social problems. The five problems and their science components are:

Problem 1. Overpopulation and its consequences: air pollution (industrial, private, chemical pollutants); water pollution (industrial, private, chemical pollutants, radioactive pollutants); strain on ecological balance; strain on resources (water, clean air, food, space); and quality of life in cities (overcrowding and aggression, noise, sanitation). The relevant science content is: human reproductive system, birth, growth, development, death, reproduction, contraception; atmosphere, composition, movement, weather, climate; conversion of energy in living things, oxidation (respiration and combustion), sun as energy source, photosynthesis; chemistry and physics of certain industrial processes, water and solutions; physiological effects of chemicals and radioactivity on plants, animals, and man; micro-organisms, decomposers of wastes, carriers of disease, transmittal of disease; the ecosystem, ecological niches, diversity of species, natural selection; cycles, carbon dioxide-oxygen, nitrogen; food chains, predator-prey relationships; man's requirements for good health, nutrition (essential elements, sources, substitutions), growth, nurture and selective breeding of food animals; conservation of natural resources, water, mineral, plant, and animals; psychological and physiological effects of noise; and social behavior of animals, aggression.

Problem 2. Everyday hazards of technological life; automobiles, electrical gadgets and tools, household chemicals, and household gadgets. The relevant science content is: physics principles (momentum, expansion, moments, levers); electrical circuits; electrical units; physiological effects of chemical agents; and, acids and bases, neutralization.

Problem 3. Biological engineering: transplants; eugenics and genetic manipulation, and drugs. The relevant science content is: human anatomy and physiology (including circulatory, digestive, nervous, reproductive, endocrine, and immunological systems); cell physiology; and genetics and genetic code.

Problem 4. Extraterrestrial exploration: feasibility and extent; necessity and costs. The relevant science content is: Newtonian physics; propulsion of space vehicles (chemical fuels, nuclear energy); geography of solar system; biological needs of man in space.

Problem 5. War and disarmament: massive nuclear assaults; detection and surveillance; chemical and biological weapons. The relevant science content is: nuclear physics; physiological effects of radiation and radioactive isotopes; electronic systems; effects of chemical and biological agents on physiology of plants, animals, and man.

The content of a few units does not appear on this list. These are the units in the early part of the program which deal with the process skills of observing and measuring. There is something quite interesting about the science content list. At least half of the topics listed are related to topics which the child most frequently asks questions about. This suggests that most of the science content selected for inclusion in the program has relevance both to the social circumstances of today and to the interests of the individual child.

Our third concern stems from one of the consequences of living in a technological age where change is rapid. The continued advent of new technologies has brought about an alteration in the traditional pattern of occupations and careers. No longer does a person choose a career specialty as a youth, train for it in school, and pursue it throughout his life. Rather, the career of a young man

or woman starting to work in the 1980's is likely to include several major occupational shifts, as specific occupations are made obsolescent by technological change. Even within a line of work, there will be few jobs that are not periodically affected by innovations. These characteristics of occupations and careers will require that the individual often learn new skills and tasks and acquire new knowledge. He will be a learner for much of his life, not only during the years of formal schooling. For this situation, the best preparation the school can offer is to help the child become an effective independent learner. Consequently, a science program which is relevant to contemporary social circumstances must make provisions for the student to learn how to learn. This can be done by including in the program's goals not only the customary objectives which pertain to achievement in science subject-matter content and in scientific inquiry, but also objectives for developing the student's skills in self-direction and self-evaluation. While the development of these skills should be a commitment of all school programs in these times, it is altogether fitting to stress self-direction and self-evaluation in a school science program. In science itself, the investigator is obliged to direct his own work and to judge its worth. Self-direction and self-evaluation are a part of inquiry in science. By stressing these same qualities in a science program, we are doing no less than emulating the scientific enterprise, and thereby providing the student with a perspective of scientific inquiry.

On the basis of the three concerns I have just reviewed, we have delineated a set of five goals for the *Individualized Science* program.

I. Student self-direction goal. The student views the learning process as primarily self-directed and self-initiated.

II. Student co-evaluation goal. The student plays a major role in evaluating the quality, extent and rapidity of his learning.

III. Affective goal. The student displays positive attitudes toward his study of science, scientific inquiry, and the scientific enterprise.

IV. Inquiry goal. The student is skillful in using the processes of scientific inquiry and is able to carry out inquiries.

V. Scientific literacy goal. The student acquires a foundation of scientific literacy.

Some comments on our perception of scientific literacy may be appropriate at this point. Four components are encompassed in the notion of scientific literacy. First is the behavioral component implied by the common literal meaning of literacy, that the student can describe his observations and experiences with terms drawn from his basic vocabulary of science words. A more fundamental component of scientific literacy is the student's understanding of certain important concepts, principles, and conceptual schemes of science. We consider a scientific idea important if it is relevant to the child in orienting himself to the world, both the natural world and contemporary society. A third component of scientific literacy concerns the student's realization that scientific ideas change over time and his understanding of other key ideas related to the nature of scientific inquiry. The fourth component, last in this list but not the least important component of scientific literacy, is the student's understanding of significant ideas related to the social aspects of science. We believe that a

foundation of scientific literacy encompasses all four of the foregoing components.

The goals listed above represent a kind of composite description of a student near the end of his *Individualized Science* program. The student's attainment of any or all of these goals is, of course, a developmental process. We assume that there are identifiable levels in the student's development toward the attainment of the desired goals. We also assume that it is possible to specify most of the student's behaviors related to these five goals at each developmental level in terms of competencies he has attained. In *Individualized Science,* there are ten levels of student development, Levels A through J. The approximate current school grades which correspond to these ten development levels are: A, K-2; B, K-3; C, 1-4; D, 2-5; E, 3-6; F, 4-7; G, 5-8; H, 6-8; I, 6-8; and J, 7-8. Since the student's progress in the program is an individual matter, there is no exact correspondence between the number of years he has spent in school, i.e., school grades, and his developmental level in *Individualized Science.* In the first three levels, A through C, the child is introduced to various processes of scientific inquiry, and he has many opportunities to sharpen his skills in using these processes as he explores in several different areas of science. In the next four levels, corresponding approximately to the intermediate grades, the main focus is on the child's application of his process skills in problem solving and on his accumulation of knowledge and understanding about himself and his environment. In the last three levels, H through J, the child carries out genuine investigations, i.e., inquiries where the answers are not known. He utilizes his process skills and existing knowledge to acquire new concepts, principles, and insights.

A significant element in promoting the student's ability to direct his own learning is the opportunity to make choices. Moreover, an individualized program must make many alternatives available to the student, because different students have different interests. In designing a school program, however, a conflict arises between giving the individual free choice in what he will study and assuring that his needs are met in developing certain recognized skills and understandings. Our resolution of this conflict has been to provide a Mainstream in *Individualized Science* and, at the same time, to offer the student numerous Alternative Pathways. The Mainstream includes a common core of science learnings in which every student is expected to achieve mastery. Units of study on the Mainstream are arranged in a sequence. For example, the Mainstream organization for Levels E and F of *Individualized Science* begins with Voit and continues sequentially through Joule, Beaumont, Powell, and Harvey. Each unit is identified by the name of a scientist who made some contribution to the field of science that the particular unit concerns itself with. Thus, the Voit Unit is concerned with human nutrition, the Joule Unit with the concept of energy, the Beaumont Unit with the processes of digestion, the Powell Unit with the role of water in natural systems, and the Harvey Unit with the circulation of blood in man.

A student may choose to interrupt the continuum of these five Mainstream units at any time by selecting one or several of the activities in the Alternative Pathways. There are various possible Alternative Pathways activities available to students working in the E and F levels. They are: Linnaeus, Copernicus, Archimedes, Volta, Watt, Kepler, Audubon, Drew, and Arrhenius.

Whole units of study, such as the Watt Unit, which is concerned with the expansion and contraction of materials with changes in temperature, or the Linnaeus Unit dealing with the growth of plants from seeds, are available. Each of these Alternative Pathways units arises from possible points of curiosity on the student's part or his desire to know more about topics taken up in the Mainstream units. Other Alternative Pathways activities give the student opportunities to investigate science concepts which may or may not be related to the subject-matter content of the Mainstream units.

The notion of Mainstream and Alternative Pathways is used, not only at Levels E and F as illustrated, but throughout *Individualized Science*. As the student progresses, he has continually increasing opportunities to select learning experiences in diverse and meaningful Alternative Pathways. Within the units themselves, choices are also offered to the student. In each unit, there is generally a common core of learnings, developed through a sequence of indi-

TABLE 1.—Learning Resources of *Individualized Science*.

LEARNING RESOURCES		Contribute to the attainment of				
		Student Self-Direction Goal	Student Co-Evaluation Goal	Affective Goal	Inquiry Goal	Scientific Literacy Goal
Individual Lesson	(IL)		X		X	X
Individual Taped Lessons	(ITL)				X	X
Men & Ideas Filmstrip	(M&I)			X		X
Mini-Exploration	(MEX)	X	X	X	X	X
Mini-Investigation	(INV)	X	X	X	X	X
Directed Readings in Science	(DRS)		X	X		X
Directed Group Activity	(DGA)			X	X	X
Student Seminar	(SEM)		X	X	X	X
Student Activity	(SA)	X	X	X	X	X
Invitation to Investigate	(ITI)	X	X	X	X	X
Self-Initiated Independent Activity	(SIIA)	X	X	X	X	X
Science Learning Game	(SLG)			X	X	X
Science Books (selected readings)	(BKS)	X	X	X		X
Individual Science Journal (student publication)	(JOUR)				X	X
Guidance and Selection System	(GSS)	X	X			X
Individual Prescription	(IP)	X	X			

vidual lessons, laboratory exercises and investigations, readings, and small group discussions. In addition to the common core, a Mainstream in miniature, various alternative and additional investigations, activities, and readings are available for the student to choose from. In the units also, the student receives instruction in how to manage his learning in *Individualized Science,* through what we call a "Guidance and Selection System." This system makes it possible for the student to practice self-direction within the program. Having experienced and practiced self-direction in *Individualized Science,* the student may well become confident about directing his own learning in many other situations.

I should like to emphasize that we are committed to the realization of all five goals of *Individualized Science.* We seek to give approximately equal attention to each goal. Throughout the program, we do not intend to neglect, for example, the student's development in self-direction or inquiry in favor or promoting scientific literacy. To make possible the realization of all five goals, our principal strategy is to make available a large variety of learning resources for the student's use in the program. These learning resources are listed in Table 1. In some of the learning resources, student behaviors are emphasized that contribute to the attainment of certain goals, while other learning resources emphasize behaviors that contribute to attaining other goals.

This strategy may be illustrated by looking at some of the learning resources available in one unit from Level D of *Individualized Science.* Students working in Level D usually are either third or fourth graders. The Lavoisier Unit in Level D uses these eleven types of learning resources: Individual Lesson, Individual Taped Lesson, Men & Ideas Filmstrip, Mini-Exploration, Directed Readings in Science, Directed Group Activity, Student Seminar, Student Activity, Self-Initiated Independent Activity, Science Learning Game, Guidance and Selection System.

All of these resources make some contribution to the attainment of the Scientific Literacy Goal. The four components encompassed in our notion of scientific literacy were previously discussed. The science subject-matter of the Lavoisier Unit relates directly to the first two components, *viz.,* building a vocabulary of science words and developing an understanding of science concepts. The Lavoisier Unit deals with the concept of burning and the scientific description of burning. The unit takes advantage of the child's natural curiosity about fire to lead into a consideration of how a candle burns and how various fuels burn in air. Fuels combine with oxygen, producing carbon dioxide and water vapor, and energy in the form of heat and light is released. The content and organization of the Lavoisier Unit are indicated on a student's Planning and Recording sheet for the unit.

Contributing to the attainment of the Student Self-Direction and Co-Evaluation Goals are the GSS (Guidance and Selection System) lessons of the Lavoisier Unit. The GSS lessons, in conjunction with the Planning and Recording sheet, guide the student in choosing his learning activities and in planning for and evaluating his own learning. In the series of three GSS lessons of the Lavoisier Unit, the student takes a placement test to demonstrate what prior knowledge he has of the unit's content; he scores his placement test; he decides whether or not he has achieved mastery of the content of each of the unit's Individual Lessons; he prescribes for himself the Individual Lessons he hasn't mastered; he

NOTES

SHRUBS OF LINCOLN LOG CABIN STATE PARK, COLES COUNTY, ILLINOIS

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ABSTRACT.—Many species of cultivated shrubs at Lincoln Log Cabin State Park are reproducing and becoming a part of the local flora. One of these, *Euonymus europaeus* L. is reported as being naturalized in Illinois for the first time.

Lincoln Log Cabin State Park, located 8 miles south of Charleston, Coles County, Illinois (Sect. 21, R9E, T11N), is an 86 acre park which contains the reproduction of the last home built and occupied by Abraham Lincoln's father. Before being dedicated as a State Park, most of the area was under cultivation. Since that time, some of the park has been planted in pines and some in pasture and prairie grasses. Most of the park, however, has not been "improved" and is in various stages of succession toward the upland oak-hickory forest common to this part of Illinois.

Presently, most of the undisturbed parts of the park are in the shrub and early tree stages of succession. Associated with the native woody plants are some cultivated shrubs. Plantings of these shrubs are common along the roads and buildings of the park. Due to natural reproduction, these shrubs have become an important component of the flora of the park and in many instances are more common than the native species.

Following is a list of the cultivated shrubs that have become established by natural reproduction in and around the park. In this list the nomenclature follows that of Jones (1963) and Bailey (1949). Each species is followed by a brief note concerning its distribution and habitat in and around the park, and the collector's name and field number. Except for *Viburnum recognitum* Fern. the species are new records for Coles County according to Jones and Fuller (1955) and Winterringer and Evers (1960). All specimens are deposited in the Stover Herbarium of Eastern Illinois University.

Euonymus europaeus L. At the edge of the pine plantations, scattered in open fields that are in the shrub stage of succession, and in fence rows outside of the park. Ebinger 10957.

Physocarpus opulifolius (L.) Maxim. In second growth open woods and in fence rows on the north edge of the park. Ebinger 10946.

Rhus aromatica Ait. At the edge of the pine plantations, scattered in open fields that are in the shrub stage of succession, and in fence rows. Eginger 10950.

Rosa multiflora Thunb. Scattered throughout the park and in abandoned fields around the park. Ebinger 10947.

Viburnum lentago L. Fence rows in and around the park. Ebinger 10952.

Viburnum recognitum Fern. Scattered throughout the park. Extremely common in the pine plantations and in the second growth open woods. Ebinger 10953.

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Manuscript received January 6, 1972.

A NEW TRUFFLE RECORD FOR ILLINOIS

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ABSTRACT.—*Tuber longisporum* Gilkey is recorded for Illinois for the first recorded account of the fungus on the North American Continent since it was originally described from Ithaca, New York in 1925.

Truffles belonging to the species *Stephensia shanori* (Gilkey) Gilkey have been reported as occurring in Illinois (Uecker, 1967). The ascocarps of this truffle were collected at Urbana, Illinois by L. Shanor

in 1953. To my knowledge this constitutes the only published account of truffles in Illinois until the present report.

Ascocarps belonging to the fungus which is the subject of this note were collected at Lake Argyle State Park, McDonough County in 1971. The identification of the fungus, *Tuber longisporum* Gilkey, was determined by J. M. Trappe. The description of *T. longisporum* by Gilkey (1925) was based on specimens collected at Ithaca, New York by H. H. Whetzel on July 20, 1903.

One of the fruiting bodies (EFM 1406) was discovered by H. H. Edwards while he was in the process of digging for earthworms on July 18, 1971. The remaining ascocarps (EFM 1407) were uncovered by H. H. Edwards, G. W. Eslinger and the writer on July 20, 1971 in approximately the same area. The specimens were taken from a depth of no more than two or three inches and some were partially exposed from the soil but covered by heavy leaf litter. The area was dominated by oak trees.

The ascocarps were light brown when fresh but have darkened somewhat after preservation in a 30-3 (ethanol-formalin) solution. The largest ascocarp is about 2 cm in diameter and they range in size down to .8 cm in diameter. For the most part, they are subspherical to very irregular in shape.

The asci generally contain 4 ascospores, however many asci contain from 1-3 ascospores and occasionally 5 are found in an ascus. The alveolate nature of the mature ascospores is most evident. The spores are mostly ellipsoidal and range in size from 24 x 44 μ to 28 x 50μ (fig. 1). They are yellow becoming light brown with age.

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Manuscript received February 7, 1972.

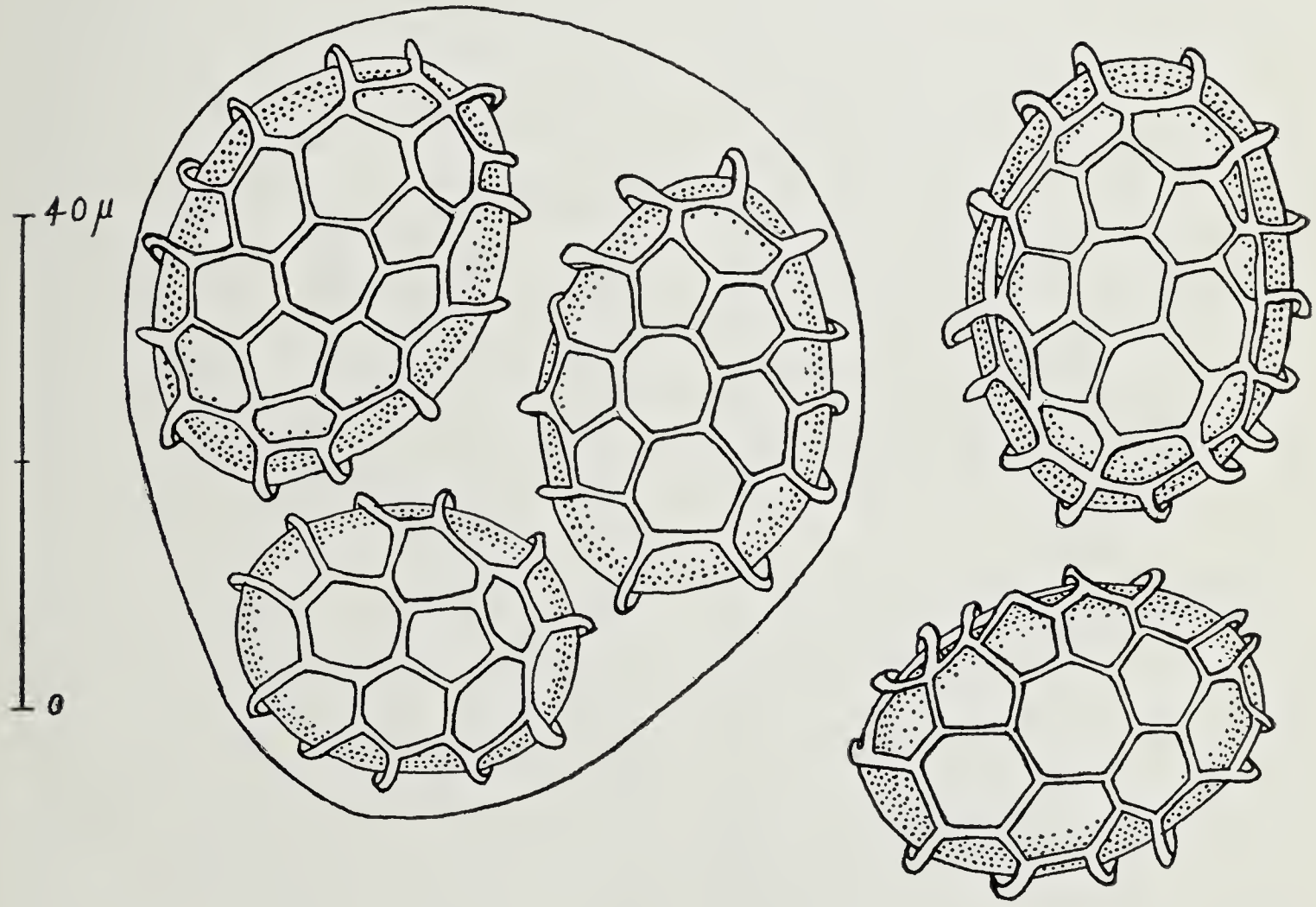

Figure 1.—*Tuber longisporum* Gilkey. Ascus and ascospores.

NEW NORTHEASTERN ILLINOIS LOCALITY RECORDS FOR THE WESTERN HARVEST MOUSE *(REITHRODONTOMYS MEGALOTIS DYCHEI)*

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ABSTRACT.—Northeastern localities of *Reithrodontomys megalotis* are reported for Will, Dupage, and DeKalb Counties. Its distribution and movement patterns within Illinois are discussed.

Pinkham and Meade (1970) summarized the distribution of *R. megalotis* in Illinois, citing 16 localities, and adding two additional locations in Champaign County. The present known localities, including our findings are shown in Figure 1. Between 16 and 29 June, snap traps were set and once a week between 7 and 29 July, traps were set in a prairie habitat along a railroad. The location bordered U.S. route 30, 10 miles N.W. of Plainfield, Will County, Illinois. A total of 5400 trap-nights were set. Three *R. megalotis,* two males and one female, were caught during the June trapping period. All three specimens were trapped within twenty meters of each other in a stand consisting largely of bromegrass (*Bromus inermis*) and interspersed with patches of osage orange (*Maclura pomifera*).

Measurements from the two males (NIU 933, and HDW 1149) are 135, 69, 18, 13 and 120, 64, 15.6, 12.2 mm for total length, tail length, hind foot, and ear from notch respectively. The skull measurements were. 20.5, 31, 9.5, and 20.4, 9.5, 10.0, 9.5 mm for total length, interorbital width, zygomatic breadth, and breadth of brain case respectively. The external measurements for the females (NIU 932, skull destroyed) are 129, 59, 15, 11. On the basis of coloration, distribution, and measurements these specimens were assigned to the subspecies *R. m. dychei,* (Hoffmeister and Warnock, 1955 and Jones and Mursaloglu, 1961).

Evidence for the presence of the Western harvest mouse in DeKalb County comes from owl pellet data. A collection of 20 pellets were gathered from a cultivated stand of various coniferous species on 15 May, 1971, 2½ mi. North of DeKalb, Illinois along Rich Road. The pellets were deposited by a long-eared owl (*Asio otus*) which appeared to be a resident in that stand at that time. One of the pellets contained the remains of one skull of *R. megalotis.* Since we have no information on the range of this individual owl, we cannot know the original source of the skull. However, the owl was sited at later times in this stand and there were 20 pellets collected and numerous scattered remains of decomposed owl pellets. These facts indicate that the owl was a resident in this stand and not just a transient or a recent immigrant.

Mr. Robert H. Satterfield collected five specimens of *R. megalotis* in a railroad prairie in DuPage County, Barrington, Illinois in 1962 (personal communication to Harlan D. Walley). These specimens are currently being deposited in the NIU collection. These localities in Will and DuPage counties extend the present localities approximately 90 miles N.E. of the closest known localities in Stark, Whiteside, and Carroll Counties.

Previous collectors of the harvest mouse in Illinois have emphasized the

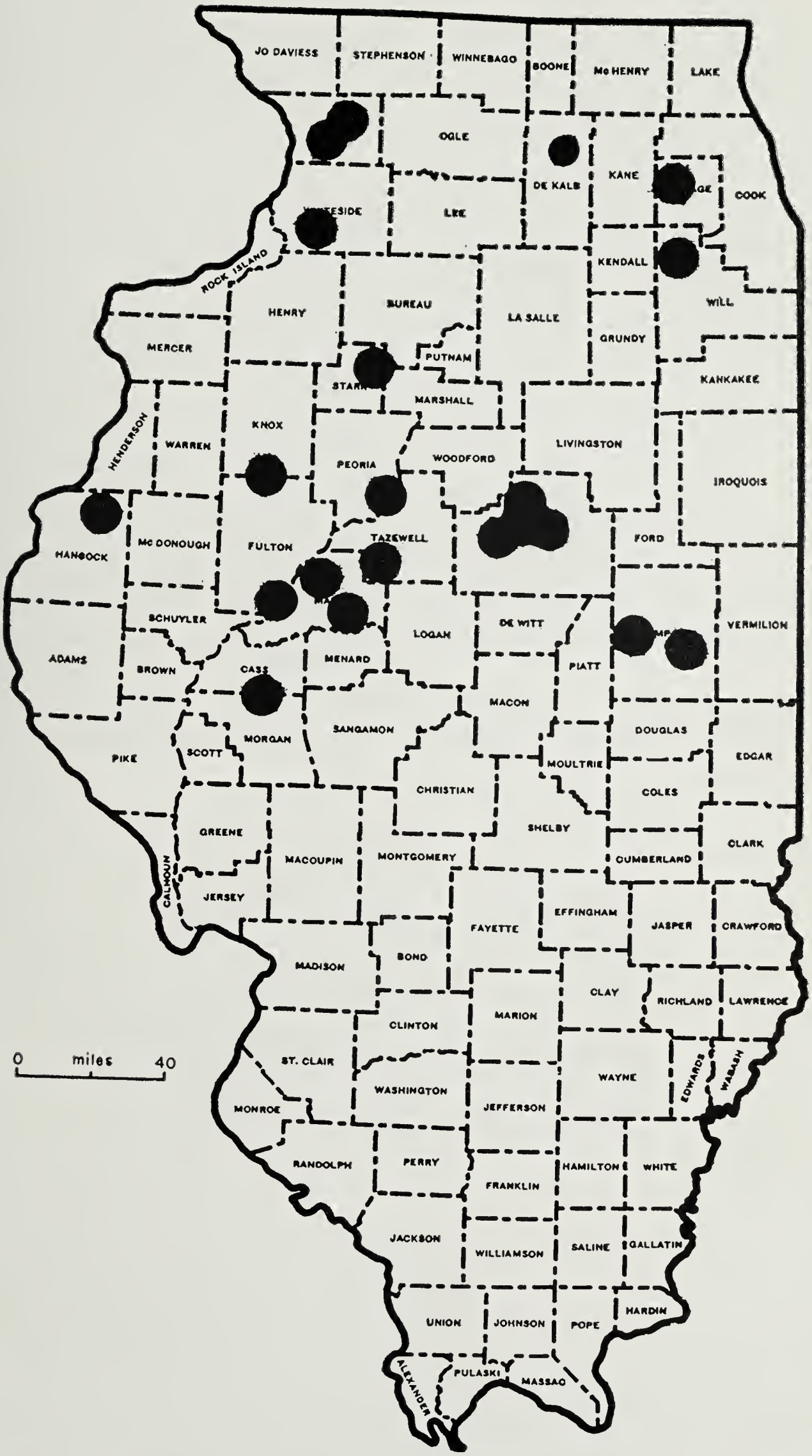

FIGURE 1.—Map showing current records for *Reithrodontomys megalotis* in Illinois.

extreme number of trap nights required in obtaining specimens, and have also mentioned the sporadic occurrence of this species in this state. Birkenholz (1967) suggested that the dispersion of the harvest mouse in Illinois follows the alterations of the habitat by man. These alterations include the clearing of woodland and maintenance of prairie habitat by burning along railroads. However, it is very difficult to make judgments on its true range extention just on the basis of new locality data of a species that is rare and sporadic in its distribution. *R. megalotis* probably ranges sporadically throughout the northern portion of Illinois, and will likely be found in rare numbers throughout the prairie peninsula of Illinois and extensions of the prairie created by farming and railroad rights of way.

The authors wish to thank Mr. Harlan Walley for his aid and comments on these findings. This research was supported by the National Science Foundation Student Originated Studies grant no. GY-9135.

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Manuscript received November 15, 1971.

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